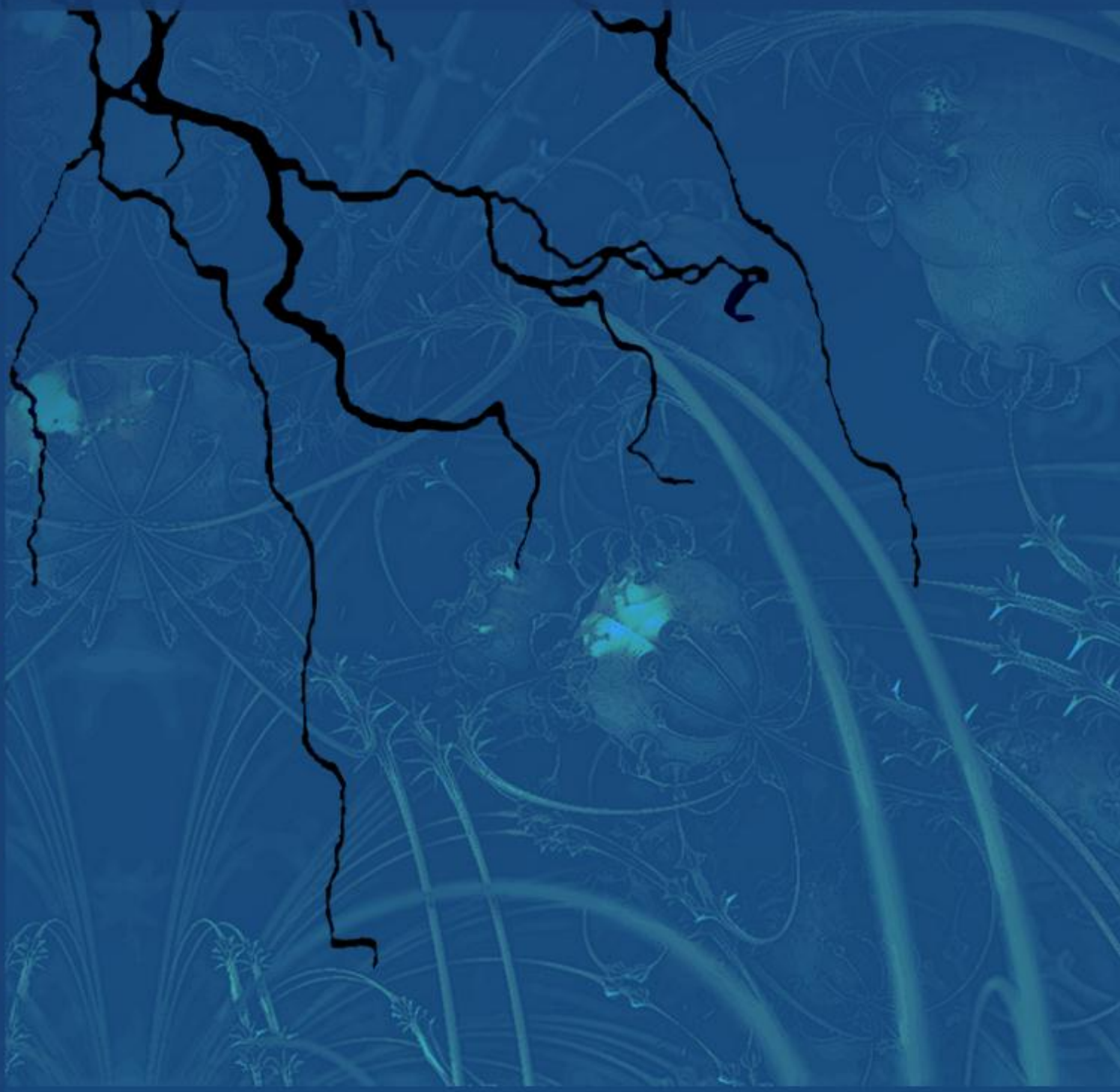
# Optimizing the plant microbial fuel cell: diversifying applications and product outputs

Jan Arends



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2013

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# Optimizing the plant microbial fuel cell: diversifying applications and product outputs

Jan B. A. Arends MSc.

Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Titel van het doctoraat in het Nederlands: Optimalisatie van de plant microbiële brandstof cel: diversificatie van de toepassingen en de producten.

Cover illustration by Viveka Toetenel

Please refer to this work as:

**Arends J.B.A.** (2013) Optimizing the plant microbial fuel cell: diversifying applications and product outputs. PhD thesis, Ghent University, Belgium.

ISBN: 978-90-5989-672-7

This work was supported by the European Community's Seventh Framework Programme FP7/2007-2013 under Grant Agreement No. 226532 "PlantPower'.

| | |
|---|---|
| 1,2 DCA | 1,2 Dichloroethane |
| AD | Anaerobic Digestion |
| AEM | Anion Exchange Membrane |
| AR | Application Ratio |
| ARB | Anode Respiring Bacteria |
| AQDS | antraquinone-2,6-disulfonate |
| BES | Bioelectrochemical System(s) |
| Bio-Pd | Bio-Palladium |
| BFC | Biological Fuel Cell |
| C | Coulombs |
| CFC | Chemical Fuel Cell(s) |
| CFU | Colony Forming Units |
| CHP | Combined Heat and Power unit |
| CIP | Cleaning In Place |
| Co | Community Organization |
| COD | Chemical Oxygen Demand |
| CEM | Cation Exchange Membrane |
| CV | Cyclic Voltammetry |
| DGGE | Denaturing Gradient Gel Electrophoresis |
| $DiaH_3$ | de-iodated Diatrizoate: 3,5-diacetamidobenzoate |
| $DiaI_3$ | Diatrizoate |
| DSA | Dimensionally Stable Anode |
| DW | Dry Weight |
| $E_{an}$ | Anode potential |
| $E_{cath}$ | Cathode potential |
| EET | Extracellular Electron Transfer |
| EIS | Electrochemical Impedance Spectroscopy |
| GDE | Gas Diffusion Electrode |
| HER | Hydrogen Evolution Reaction |

| | |
|---|---|
| I | Current |
| J | Current density |
| KVE | Kolonie Vormende Eenheden |
| LCA | Life Cycle Assessment |
| LSV | Linear Sweep Voltammetry |
| MEC | Microbial Electrolysis Cell(s) |
| MES | Microbial Electrosynthesis |
| MFC | Microbial Fuel Cell |
| MRM | Microbial Resource Management |
| OCV | Open Circuit Voltage |
| PI | Propidium Iodide; a red fluorescent dye |
| PMFC | MFC containing a plant |
| qPCR | quantitative Polymerase Chain Reaction |
| rRNA | ribosomal RiboNucleic Acid |
| R | Resistance |
| Rr | Range weighted Richness |
| RVC | Reticulated Vitreous Carbon |
| SEM | Scanning Electron Microscopy |
| SG | Sybr Green I; a green fluorescent dye |
| SHE | Standard Hydrogen Electrode |
| SMFC | MFC with the anode in soil or sediment |
| TCE | Trichloroethene |
| VFA | Volatile Fatty Acid |
| V | Volt |
| vs. | versus |
| VS | Volatile Solids |
| VSS | Volatile Suspended Solids |

# Table of Contents

# CHAPTER 1: INTRODUCTION TO BIOELECTROCHEMICAL SYSTEMS

This chapter has been redrafted after:


Arends JBA, Desloover J, Puig S, & Verstraete W (2012)
Principles and technology of microbial fuel cells. *Fuel Cell Science and Engineering*, eds Stolten D & Emonts B (Wiley-VCH Verlag GmbH & Co. ), pp 147-184.

Arends JBA & Verstraete W (2012)
100 years of microbial electricity production: three concepts for the future. *Microbial Biotechnology* 5(3):333-346.

Desloover J, Arends JBA, Hennebel T, & Rabaey K (2012)
Operational and technical considerations for microbial electrosynthesis. *Biochemical Society Transactions* 40(6).

# 1. Challenges to global water, food and energy supply

Mankind currently faces several challenges for the global society to stay in a business as usual mode of living (Jones & De Meyere, 2009). It can be considered that the global society is and has always been in a state of flux. However from a western society perspective, the last few decades were fairly stable in terms of food and energy supply (there was plenty available) and environmental conditions (Water, Air and Land pollution continuously declining in Europe). Nowadays, with an ever more connected global society it becomes clear that this relatively stable situation is not stable and does not apply to the vast majority of the global society. For the introduction of this work, four areas (water, food, energy and environment) that need considerable and urgent attention the coming decades in order for mankind to meet its needs will be briefly addressed. As the global human population grows and strives for a higher standard of living, the pressure on clean water sources, energy and food supply and the natural environment becomes even larger. The challenges concerning these four areas can hopefully be (partially) tackled by implementing technological solutions. However technological solutions will never be successful by themselves, they need to find backing by societal acceptation and implementation.

**Clean water**

Access to clean water is essential for human and animal health. Water is an essential food source that is becoming more and more scarce. Only 1 % of the total water content of the earth is fresh, accessible and clean enough to use for consumption (Un-Habitat, 2010). Whereas the worlds fresh water supply remains the same, population growth leads to lower availability per capita. An estimated 3.2 billion people will have to make due with 2.7 L $d^{-1}$ of clean water in 2025. This number increases to 4.9 billion in 2050 (over ½ the estimated world's population). Technological solutions to the water challenge include wastewater treatment, re-use of water for non-potable uses, closed water cycles in production processes, desalination of seawater etc. However, access to these technologies is often limited or non-existent for a large part of the global population (Unep, 2008; Un-Habitat, 2010).

**Stable and sustainable energy supply**

Next to water, energy is in high demand for the same reasons as mentioned above. Energy has been generated by means of consuming fossil fuels (coal, oil & natural gas), making use of nuclear reactions and from renewable sources (wind, solar, water, thermal, fuel crops). Non-renewable energy supply is not only threatened by the fact supplies are limited but also due to environmental and geopolitical considerations. For example, harvesting oil, coal and natural gas comes at a certain price for the natural environment (see for example oil spills with the Exxon Valdez in Alaska, illegal and primitive oil mining in Nigeria and the spill with Deepwater Horizon in the Gulf of Mexico, etc. ). The remaining energy supplies are not equally distributed around the globe thus not every nation has access and not everyone is willing to share. All three reasons have caused a rise of investment in renewable energy production and has driven the increase of a 'bio-economy' with corn-ethanol, anaerobic digestion, biodiesel as main examples.

**Food supply**

Based on the same arguments as are valid for water and energy supply, food supply is also a major challenge for mankind. It was estimated in 2005 that 1.4 billion people were living in extreme poverty (less than $ 1.25 to spend per day) (Unep, 2009). Not enough clean, pathogen free water is a major challenge for sustainable crop production (Un-Habitat, 2010). The rising demand for energy crops is another challenge for the global food supply. Any arable land that is used for the production of energy crops is lost to the production of food crops (Unep, 2009).

**A clean environment**

A clean natural environment is a fourth essential prerequisite for life to thrive. A clean environment is difficult to define but water, air and soil pollution lead to loss of life quality for humans and animals. Food and fuel production using current common practice leads to various polluting activities such as greenhouse gas emissions ($CO_2$, $CH_4$, $N_2O$) by combustion of fossil fuels, rice cropping, livestock breeding and land fertilization. Moreover, organic and inorganic (micro)pollutants end up in freshwater bodies causing algal blooms, growth of (opportunistic) pathogens, disturbance of natural ecosystems (for example the influence of discharged hormones on fish sex distribution) (Un-Habitat, 2010).

It is clear that humanity faces different challenges that are interconnected, which calls for integrated and novel technologies to tackle these. In this work, the focus is on so-called microbial bioelectrochemical systems (BES), in which microbial catalysts are used to drive electrode reactions. A BES allows for energy efficient (waste)water treatment, electricity driven production of inorganic molecules ($H_2O_2$) and fixation of $CO_2$ to fuels, bioremediation…, currently all at lab-scale. In the coming chapters the mode of action of BES is discussed, and then further deployment of BES the vicinity of plants to obtain novel water, energy and bioproduction processes will be highlighted.

## 2. Bioelectrochemical systems

Microbial fuels cells (MFC) or as they are called nowadays microbial bioelectrochemical systems (BES) have been described over 100 years ago. Ever since Potter (1911), inspired by the findings of other biologists in the late 19th and early 20th century, devised the first MFC (although he did not call it a such), these devices have seen a long way of research and development. A MFC can be distinguished from chemical fuel cells (CFC) or batteries by the fact that at least one of the two reactions in the fuel cell is catalysed by a biological component (Figure 1.1) (Logan et al., 2006). Generally speaking this is the anode reaction, where organic carbon or another electron donor is oxidized by microorganisms while a solid-state electron acceptor (i.e. the electrode) is subsequently reduced. However, microbial catalysed cathode reactions are developed more and more. The microorganisms present on the cathodic electrode are able to receive electrons from a solid material and subsequently reduce a final electron acceptor. Nutrients for growth and maintenance are obtained from the liquid or gas phase.

Regarding the naming of these devices, different names have been coined. A microbial fuel cell (MFC) designates a fuel cell where one or both reactions are microbial catalysed. In plain terms a MFC can be likened to a battery powered by bacteria and not chemicals. Not only whole microorganisms are able to catalyse redox reactions, also enzymes can have a catalytic function in a fuel cell as well (Ivanov et al., 2010). The focus of this work will be on microbial catalysed reactions. A biological fuel

cell (BFC) idescribes a system where whole microorganisms and/or purified enzymes are used to catalyse a reaction. A microbial electrolysis cell (MEC) is a system where no power is harvested. Instead, some extra potential is applied to electrons coming from the anode to perform a reaction that under ambient conditions, based on thermodynamics, is not feasible. It is counter intuitive to supply energy to a fuel cell but in this case the purpose is not producing electrical power, but creating a product at the cathode such as hydrogen ($H_2$) or methane ($CH_4$) gas, caustic soda (NaOH), hydrogen peroxide ($H_2O_2$) or small organic acids (i.e. acetate and propionate) or other organic compounds. A MDC denotes a microbial desalination cell where a third, middle, compartment is installed that can be filled with salt water to be desalinated by the electrical field (Cao et al., 2009). SMFC and PMFC denote a sediment-MFC and a Plant-MFC respectively (De Schamphelaire et al., 2008). These particular differentiations will be further detailed in paragraph 3. As the emphasis of MFC research has shifted from power output to other functions or applications, the general term bioelectrochemical systems (BES) will be used further in this text, unless where a specific application is discussed.

In contrast to chemical fuel cells , biological fuel cells are more delicate as extreme operating temperatures ($4\ ^0C < T_{optimal} > 60\ ^0C$) and pressures (> 1 bar) can have an adverse effect on the biological catalysts. Microbiological life has been described above these thresholds, up to 121 $^0$C and 110 MPa (Kashefi & Lovley, 2003; Lauro & Bartlett, 2008) but these microorganisms are not abundantly found. Although no BES has been described under these conditions yet, some thermophilic organisms are able to respire with metal oxides at 100 $^0$C, showing opportunities to work at higher temperatures (Kashefi et al., 2008).The highest temperatures for MFC described so far are in the range of 60 °C (Wrighton et al., 2008). Some of the same materials and even the same reactants can be applied in a BES as in a CFC. For the electrodes, materials such as carbon, stainless steel, palladium and platinum are often used (Paragraph 2.1). Electron donors and acceptors used in a CFC can also be used in a BES, i.e. hydrogen and methanol as anodic reactants, while oxygen is most frequently used in the cathode. Hydrogen and small organic compounds can also be used in a biologically catalysed anode, whereas oxygen is a suitable reactant for a biologically catalysed cathode. Next to these small molecules, heterogeneous mixtures of larger molecules or recalcitrant compounds can also be used as electron donors and acceptors in a BES.

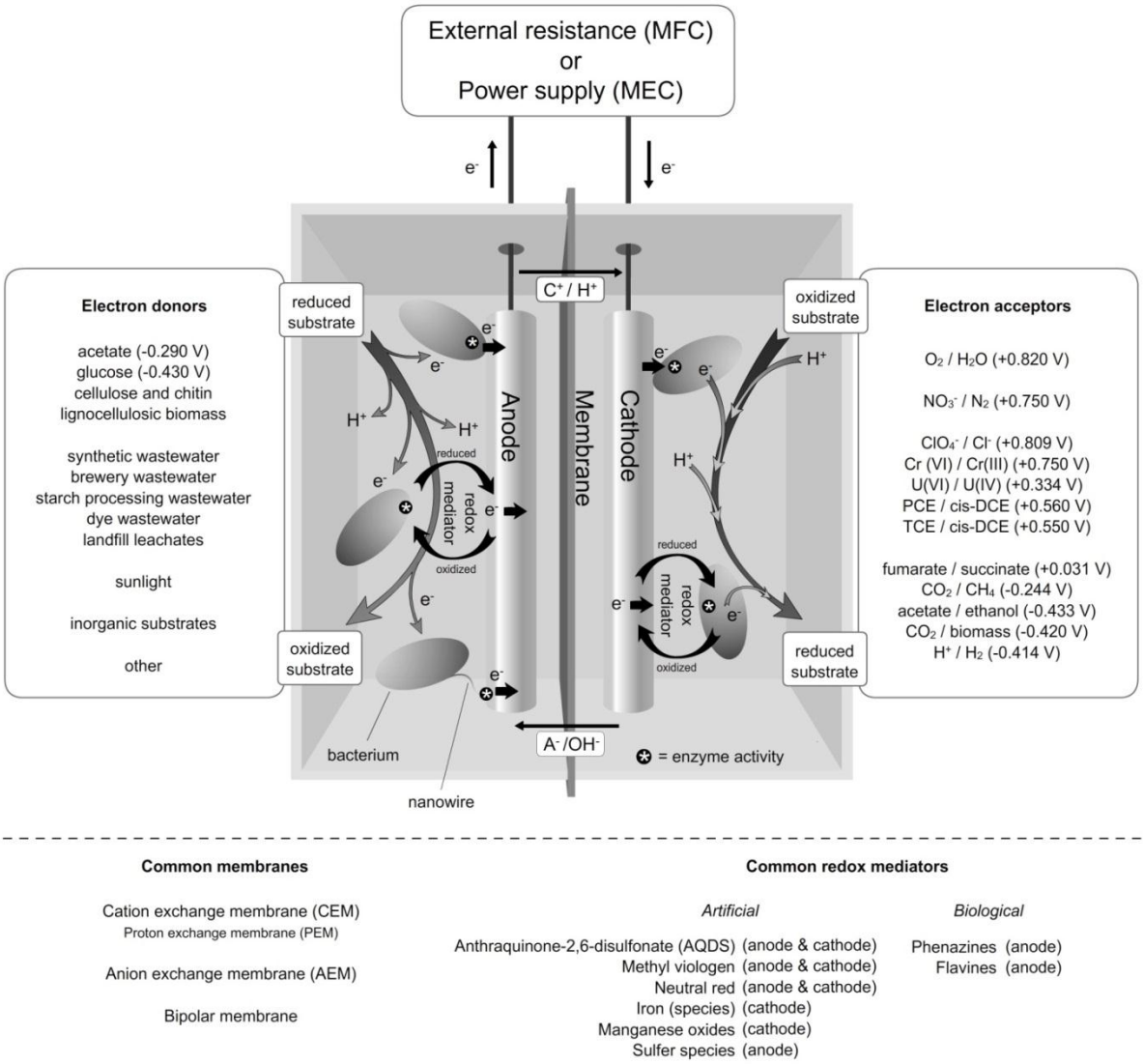



***Figure 1.1:*** *Overview of a Bioelectrochemical system (BES) with examples of electron donors and acceptors, membranes and redox shuttles. MFC: microbial fuel cell, MEC: microbial electrolysis cell, $A^-$: anion, $C^+$ cation, $e^-$: electron. In MFC mode, power can be harvested. In MEC mode, power is invested to drive a reaction at the cathode. Sunlight is mentioned as an electron donor, although the electrons are derived from water ($2\ H_2O \rightarrow 4\ H^+ + 4\ e^- + O_2$), sunlight is driving the liberation of electrons. Artwork by Tim Lacoere, LabMET, Ghent University.*

## 2.1 Microbial electrocatalysts

Microbial electrocatalysis relies on microorganisms as catalysts for reactions occurring at electrodes. The microorganisms involved are able to transport electrons in and out of their cell, a process known as extracellular electron transfer (EET), and can catalyse both oxidation and reduction reactions (Thrash & Coates, 2008; Rabaey & Rozendal, 2010). Their catalysing properties have been confirmed by the fact that they are able to lower the overpotentials (lower energy loss) both at anodes (Lowy et al., 2006) and cathodes (Rabaey et al., 2008; Ter Heijne et al., 2010a), causing an increased performance of the system. Nevertheless, they cannot be considered as true catalysts since part of the substrate/electrons is consumed for growth and cellular maintenance.

### 2.1.1 Anode biocatalysis

In bioanodes, an (in)organic electron donor is oxidized by microorganisms with concomitant liberation of electrons and protons (Figure 1.1). The produced electrons are shuttled through the internal electron transport chain of the microorganisms and are deposited on the anode (Richardson, 2000; Shi et al., 2009). The energy level of the electrons deposited on the electrode is dependent on the terminal electron transfer molecule. This also determines the amount of energy that an organism can use for its own metabolism and which amount is available for work. Two strategies for energy conservation by microorganisms are also being used in bioanodes. *Shewanella* spp. make use of substrate level phosphorylation while *Geobacter* spp. make use of oxidative phosphorylation for energy conservation. Electrons are transferred through a cascade of cytochromes, quinones and other electron transfer molecules from the electron donor through the inner cell membrane and periplasmic space until they finally reach a terminal electron transfer molecule in the outer cell membrane (Richardson, 2000; Shi et al., 2009; Bird et al., 2011). This is the case for gram-negative microorganisms i.e. organisms containing an outer cell membrane and a low amount of peptidoglycan. The electron transfer mechanisms for gram-positive bacteria i.e. containing a large peptidoglycan layer in their cell wall have not yet been investigated to such a detailed level.

Electron donors

The potential practical applicability of BES technology can be envisioned since different types of (waste)waters have been found suitable to drive electron donating reactions (Figure 1.1) (Pant et al., 2010a). Furthermore, next to organic electron donors, also inorganic waste streams for example containing sulphides can be used as a substrate for the anode (Rabaey et al., 2006). Sunlight has been reported as an energy source for BES. The latter requires the need of photosynthetic active plants or microorganisms, and has the additional advantage that $CO_2$ is fixed from the atmosphere (Strik et al., 2011). Sunlight as an energy source in anodes of a BES makes uses of the photosynthetic machinery to liberate electrons from water. As shown with inhibitor molecules, these inhibit various steps of the photosystem, electrons are diverted from the plastoquinone pool via cytochrome bd quinol oxidase to the anode. However the exact mechanism of terminal electron transfer is not yet know (Pisciotta et al., 2011).

Attention has to be paid regarding the type of substrate used and the loading rate as it influences the composition of the microbial community, but also influences overall performance, including power density and coulombic efficiency (Aelterman et al., 2008b; Chae et al., 2009).

Biocatalysis

A lot of research has been done to resolve the key players in bioanode processes, i.e. electrochemically active microorganisms capable of respiring with non-soluble materials (also called electricigens or anode respiring bacteria; ARB). Although a specialist microorganism *Geobacter* spp, are frequently found in bioanodes, microbial community analysis has revealed a broad spectrum of microorganisms capable of living in anode biofilms and (possibly) interacting with electrodes, comprising both gram-positive and gram-negative bacteria (Lovley, 2006; Logan, 2009; De Schamphelaire et al., 2010b). *Shewanella* spp. are frequently used to study bioanode processes (Hartshorne et al., 2007; Newton et al., 2009; Bretschger et al., 2010) although they are not commonly found in bioanodes inoculated with natural samples. Biofilms on anodes are typically in

the range of 10-50 µm thick, although cases of even thicker biofilms have been reported (Figure 1.2) (Lee et al., 2009). Bacteria densities within biofilms are difficult to quantify due to the amorphous nature of the various electrodes materials. Values up to 50 gVS $L^{-1}$ biofilm have been reported (Lee et al., 2009).

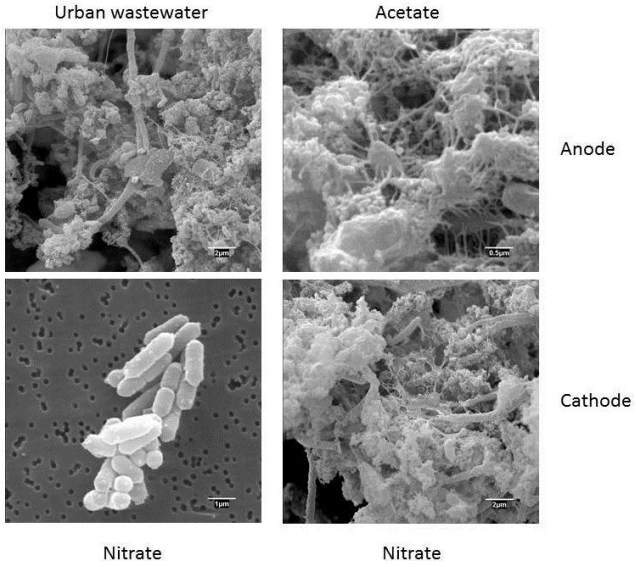



***Figure 1.2:*** *SEM pictures biofilms on electrodes. 2 anodes (top); operated with different electron donors. Filaments interconnecting the microorganisms can be clearly seen in the top right picture. They might be nanowires but their function cannot be established from this analysis only. 2 cathodes (bottom) with nitrate as the electron acceptor. Picture courtesy of S. Puig, University of Girona, Spain.*

Electron transfer mechanisms

One of the most intriguing aspects regarding these biocatalysts is the way that these microorganisms shuttle electrons to the anode. Several electron transfer mechanisms can take place, either directly or indirectly (Richardson, 2000; Rabaey et al., 2004; Shi et al., 2009; Bird et al., 2011). Furthermore, all these mechanisms can occur simultaneously within a mixed microbial community in an attempt to maximize the use of the substrate present for microbial benefit. Further analysis of the microbial community revealed that gram-negative bacteria have been shown to have much stronger EET capacities compared to gram-positive bacteria (Rabaey et al., 2005b; Milliken & May, 2007; Wrighton et al., 2008). As a result, all the proposed transfer mechanisms are based on detailed studies with gram-negative strains that have been found frequently in the microbial community of BES.

Direct electron transfer mechanisms

Direct EET can be defined as the transfer of electrons to the electrode without the need of a mobile component able to diffuse to and from the microorganism for electron transport (Rabaey et al., 2004). This requires thus a physical contact between the bacterial cell membrane and the electrode surface. Current models for direct EET are based on pure culture studies of Gram-negative bacteria like *Geobacter sulfurreducens* (Caccavo et al., 1994; Bond & Lovley, 2003) and *Shewanella oneidensis* (Gorby et al., 2006; Ringeisen et al., 2006; Rosenbaum et al., 2010a), which are both dissimilatory metal reducing organisms. Up till now, several putative pathways have been proposed (Lovley, 2006; Lovley, 2008; Torres et al., 2010; Lovley, 2012). They involve at least a series of periplasmatic and

outer membrane complexes, of which cytochromes seem to play a pivotal role (Busalmen et al., 2008). Regarding *Geobacter* spp., OmcS and OmcZ seem to be the most important electron transferring cytochromes in the final electron transfer step (Richter et al., 2009; Leang et al., 2010; Inoue et al., 2011). Additionally, type IV pili, which are appendages of the cell wall, also seem to be involved in the transport of electrons (Reguera et al., 2005). These so called nanowires have also been proposed as possible electron transport mechanisms between different microorganisms as well (Gorby et al., 2006; Morita et al., 2011) and as a means for oxic metabolism in anoxic sediments (Nielsen et al., 2010). From a practical point of view, direct electron transport eliminates to a certain extent diffusion limitations inherent to indirect transport, and simplifies solid/liquid separations as the biocatalyst can be immobilized in the reactor (Rabaey & Rozendal, 2010).

Indirect electron transfer mechanisms

Indirect EET involves the use of electron shuttles which transfer electrons from the microbial cell to and from the electrode (Rabaey et al., 2005b; Marsili et al., 2008). Examples of applied electron shuttles are humic substances like AQDS, neutral red and methylene blue. The addition of a chemical shuttle can be expensive, toxic and prone to wash-out of the system (Thrash & Coates, 2008). Next to artificial redox mediators, some microorganisms are able to produce their own mediators such as secondary metabolites like phenazines (Rabaey et al., 2005b; Pham et al., 2008b) and flavins (Marsili et al., 2008). Finally, also primary metabolites like sulfur species (Dutta et al., 2009) and hydrogen gas (Schroder et al., 2003) are able to convey electrons to and from electrodes.

Overall, mediators are able to enhance the electrical interconnectivity between electrochemically active microorganisms and the electrode, and hence increase the active range of the electrode beyond the biofilm into the bulk liquid.

### 2.1.2. Cathode biocatalysis

In cathodes, the electrons delivered by the anodic oxidation reaction are used to drive reduction reactions. Cathode reactions in BES can be either purely chemical or biologically catalysed. Typical chemical cathode reactions used in BES are oxygen (Zhao et al., 2006) and proton reduction (Rozendal et al., 2006b; Call & Logan, 2008) to water and hydrogen gas, respectively. Hexocyanoferrate is a chemical electron acceptor commonly used in cathodes (Park & Zeikus, 2000). The latter has been applied frequently as it allows investigating bioanode processes when a non-limiting cathode process is needed. However, the need for replenishment and possible toxic effects limits its application to lab-scale experiments. Next to chemical cathodes also biocathodes have been developed, and actually fit much better in the claimed sustainable nature of BES compared to chemical cathodes.

Biocatalysts

In biocathodes microorganisms play a pivotal role in catalysing the reduction reaction. Most of the electrochemically active bacteria in biocathodes have been reported to be gram-negative, although some gram-positive bacteria are able to play a role in the transfer of electrons to a terminal electron acceptor. This indicates that, likewise to anodic communities, there is potentially widespread capability of bacteria to catalyse cathodic reactions (Gregory et al., 2004; Cournet et al., 2010; Nevin

et al., 2011b). However in contrast to anode biofilms, cathode biofilms are usually rather thin and sometimes not even confluent (Figure 1.2).

Electron transfer mechanisms

Whereas a lot of studies report on the microbial assisted electron transfer towards electrodes (bioanodes), limited information is available on the reverse process. Especially direct microbial electron uptake from cathodes by microorganisms needs a thorough investigation.

Biocathode studies have shown that electrochemically active bioanodes may be turned into biocathodes upon changing the environmental and operating conditions (Rozendal et al., 2008a; Cheng et al., 2010). Therefore, Rosenbaum and co-workers recalled some known anodic EET mechanisms and evaluated their potential role in cathodic EET mechanisms (Rosenbaum et al., 2011). Their main conclusion was that also in cathodes both direct and indirect mechanisms take place, and are very similar to processes at the bioanode. However, the main difference is that the redox active components operate at higher redox potentials. Furthermore, it has not yet been demonstrated that biocatalysed electron transfer is a respiratory process yielding energy for the electrochemically active microorganisms (Huang et al., 2011a; Rosenbaum et al., 2011). For instance, it has been shown that biological oxygen reduction at the cathode can occur passively without active involvement of the bacterium (Freguia et al., 2010). Nevertheless, Clauwaert (2007a) hypothesised an electron transfer mechanism with concomitant generation of a proton motive force in the microorganisms in denitrifying biocathodes.

## 2.2 Pure cultures and mixed microbial communities

Both mixed and pure cultures can be used for the establishment of biological anodes or cathodes. From a practical point of view, mixed culture electrodes are more robust and resilient. With regards to performance, dedicated pure cultures are able to deliver the same output as mixed cultures (Min et al., 2005; Nevin et al., 2008). A drawback of using pure cultures is that more complex substrates are not converted. This can be done by making use of syntrophic and synergistic interactions within microbial communities that establish on or near the electrodes (Freguia et al., 2008; Parameswaran et al., 2010).

Pure cultures are ideal for studying fundamental aspects or for the catalysis of species-specific reactions. Trash and Coates (2008) gave an overview of pure cultures used in BES and address that these kind of studies will help us understanding of the means by which we can stimulate microbial communities to use electrical current. Furthermore, a completely new application would be the use of pure isolates for high-quality cathodic processes aimed at specific generation of products (Rabaey & Rozendal, 2010).

## 2.3 Biological limitations

Applying microorganisms in a technology brings along some biological limitations. First of all, proper physical parameters such as pH (around neutral) and temperature (15-30 °C) need to be maintained for good microbial metabolism. However as always, some bacteria are able to function at slightly different conditions. For instance, the microbial reductive dechlorination of perchlorate was the highest at a pH of 8.5 (Butler et al., 2010). Shear forces and hydrodynamic patterns are important physical parameter as it influences biofilm thickness and density (Pham et al., 2008a).

Secondly, biofilm thickness, structure, composition and density affect the flux of substrates and products within the biofilm. High concentrations of reaction products can bring along large changed thermodynamic conditions which have a negative impact on the performance of the system. In the case of bioanodes, higher power production was observed from thicker anodic biofilms (Nevin et al., 2008). Strikingly, the reverse effect has been observed in cathodic biofilms (Behera et al., 2010) .

Finally, even if the microorganisms are not limited by their environment, there are some intrinsic limitations associated with the microbial metabolism such as their growth rate, uptake rate of nutrients and electron transfer capacities. Certainly cathodic communities seem to encounter harsh conditions as they have to invest energy for $CO_2$ fixation in their biomass (autotrophic microorganisms) and need to form a biofilm on a negatively polarized surface.

## 2.4 Electrode, membrane materials and reactor design

BES has have not yet outgrown the laboratory, so a wide variety of materials has been investigated as electrode material. However, the most commonly used material, both for anode and cathode is (modified) carbon. Materials used for construction of the system are usually glass reactor flasks or plastic frames, both of which are, of course, non-conductive.

### 2.4.1 Electrode materials

Electrode materials for a BES require a high specific surface area in order to create a large surface area for bacterial attachment with the purpose to create a high volumetric current density. 1.3 $m^2g^{-1}$ for granules (Mersen, Wemmel, Belgium) and 0.6 $m^2 g^{-1}$ for felt materials (Alfa Aesar, Alfa-Aesar, London, UK) are commonly found in a BES. These materials have a volumetric surface area of $10^4$ $m^2$ $m^{-3}$ (Freguia et al., 2008) up till $10^6$ $m^2$ $m^{-3}$ (Mersen, Wemmel, Belgium) for granular material. Values of $4.5*10^4$ -$5.5 *10^4$ $m^2$ $m^{-3}$ can be found for the felt materials (Alfa-Aesar, London, UK and National Electrical Carbon Products BV., Hoorn, The Netherlands). When reporting specific electrode surfaces, one has to keep in mind that most surface areas are determined by means of $N_2$–gas adsorption. With three-dimensional electrodes, it is key to determine the surface available for biological interaction instead of the surface available for $N_2$ adsorption. $N_2$ adsorption depends on length scales in the order of angstroms i.e. $10^{-10}$ m whereas bacterial attachment depends on length scales of µm i.e. $10^{-6}$ m. So there is a 4 order of magnitude difference. On flat surfaces this is no issue as all surface area is bio-available, whereas on rough surfaces the $N_2$ surface might be very large while it is not bioavailable due to nm-scale pores.
Examples of frequently applied forms of carbon and stainless steel that have mostly been used as electrodes are granular, felt, cloth, brushes or solid blocks (Logan et al., 2007; Aelterman et al., 2008b; Ter Heijne et al., 2008; Zhou et al., 2011).

As carbon has a low catalytic activity by itself, various attempts have been made to increase the performance of carbon electrodes. The use and effectiveness of catalysts on an electrode depend on catalyst loading, stability and operating conditions of the electrode compartment (Zhao et al., 2006). Especially the use of undefined (waste) streams in combination with a microbial catalyst can lead to fouling of a chemical coating on the electrode. For example ethylenediamine treatment and nitric acid treatment of activated carbon felt increased power density by 25% and 58% respectively and decreased start-up time of the bioanode. This increase was attributed to modified surface characteristics of the original material (Zhu et al., 2011). Tungsten carbide modified anodes in combination with a pyrolyzed $Fe^{2+}$phthalocyanine modified cathode has also been shown to improve

both anodic and cathodic reactions respectively (Zhao et al., 2005; Rosenbaum et al., 2006). This is remarkable since tungsten is not a noble metal and thus cheaper compared to platinum. As another example, a 1000 fold increase in power density was observed when using a graphite anode doped with $Mn^{4+}$ and a graphite cathode doped with $Fe^{3+}$ atoms compared to the woven graphite control electrodes (Park & Zeikus, 2003).

Non-modified carbon and stainless steel felt were investigated for use as a biological oxygen reducing cathode. Both are suitable materials but operational parameters such as salinity and presence of a biofilm have a strong impact on the performance of the material (De Schamphelaire et al., 2010a). Granular graphite can also efficiently catalyse the oxygen reduction reaction by itself, without any (biological) catalyst (Freguia et al., 2007). Current densities (17 mA $m^{-2}$ projected cathode area) were attributed to the high specific surface area of the material. Unmodified carbon cloth, paper and sponge, unmodified graphite and reticulated vitreous carbon (RVC) were tested for application as a cathode in marine sediment fuel cells (Scott et al., 2008a). Next to the unmodified materials, also modified materials such as carbon paper coated with iron and Fe-Co tetramethoxyphenyl porphyrin (Fe-CoTMPP), platinised carbon and platinised titanium electrodes were used. From their results it could be seen that the cathode catalysed with Fe-CoTMPP resulted in a max power output of 60 $mWm^{-2}$ while the unmodified carbon sponge obtained about 38 $mWm^{-2}$. Their work showed that the choice of material is crucial as modified materials do not always outperform unmodified electrode materials (Scott et al., 2008a).

Recent advances in nanofabrication provide a unique opportunity to develop efficient materials for BES. Nanoparticles and carbon nanotubes have been used as material for electrodes in BES (Zhou et al., 2011). Nickel powders and chemically as well as biologically produced palladium nanoparticles have been used for hydrogen production in a MEC (Hennebel et al., 2010; Selembo et al., 2010; Huang et al., 2011b). Moreover, current density was improved 20-fold compared to a plain graphite anode using graphite disks with gold and palladium nanoparticles (Fan et al., 2011).

### 2.4.2 Ion exchange membranes and other separators

As in a chemical fuel cell, a membrane is also of importance for a BES. Membrane-less BES also showed promising results and can be a reasonable alternative (Call & Logan, 2008; Clauwaert & Verstraete, 2009). The main purpose of a membrane is 1) to separate reactants in order to prevent internal short circuiting while 2) providing a means of ion transport to ensure internal charge balancing between the anode and cathode reactions. Membranes used so far in BES research include dialysis membranes, cation exchange membranes (CEM), proton exchange membranes (PEM) and anion exchange membranes (AEM) (Rozendal et al., 2007; Sleutels et al., 2009b). Next to the conventional membranes also non-ion exchange separators have been investigated. These include cloth (Zhang et al., 2009), filtration membranes (Biffinger et al., 2007), bipolar membranes (Ter Heijne et al., 2006) and baked earth (Behera et al., 2010). Of the various membrane types, anion exchange membranes seem to be the better choice for decreasing internal resistance and increasing internal charge transport (Rozendal et al., 2007; Sleutels et al., 2009b). The use of a separator of any kind has the drawback that it enhances a pH gradient between the two compartments, i.e. it interferes with transport of $OH^-$ and $H^+$ ions. A difference of 1 pH unit between both compartments can lead to a voltage loss of 59 mV. This can be calculated with the Nernst equation (Logan, 2008). This lowers the effective output (MFC) or increases the effective input (MEC) voltage of a cell.

Moreover, membrane fouling and/or scaling can lead to an increased internal resistance of the cell, thereby lowering its output. Thus the use of a separator should be evaluated for each specific use.

### 2.4.3 Configurations and design

Most BES have been designed as a conventional fuel cell, i.e. two flat electrode compartments separated by a membrane. Design parameters can be adjusted from this traditional design. These parameters include electrode spacing, flow patterns, reactor volumes and electrode surface areas. H-type fuel cells are mostly used for studies on physiological parameters of the biocatalyst and not for optimizing output (Nevin et al., 2010; Rosenbaum et al., 2010a).

Next to the conventional design, tubular upflow designs have been studied to cope with high internal resistances to charge transfer (Rabaey et al., 2005c; He et al., 2006). A subset of researchers has focussed on miniaturizing a BES. This is done from the concept that smaller BES, having lower internal resistance, can deliver larger volumetric power outputs (Ringeisen et al., 2006; Biffinger et al., 2007). Miniaturization has also lead to systems that are easier to set-up and can be studied in large numbers (Hou et al., 2009; Moriuchi et al., 2009; Chen et al., 2011). Another advantage of small systems is the possibility to study pure or defined cultures in detail (Hou et al., 2009). Microfluidic devices have also been shown to be beneficial for assessing microbial response to environmental stimuli (Li et al., 2012). Most of this work has focused on the anode compartment so far.

Small mL-scale fuel cells are mostly used with membrane electrode assemblies (MEA) which means that the conductive electrode material is coated on a membrane. This has led to a one chamber design which contains the anode compartment. The cathode electrode is open to the air (Liu & Logan, 2004).

Large scale systems have also been used to study performance. These systems have a stacked configuration to allow multiple membrane electrode assemblies in a small volume (Ieropoulos et al., 2008). Due to the biological nature of microbial electrocatalysis, the phenomenon of cell reversal can occur which leads to a drop in usable output (Aelterman et al., 2006a; Oh & Logan, 2007). In a normal stack operation, current flows from anode 1 to cathode 1 to anode 2 etc. Stack reversal occurs when one half cell is not able to deal with the current in the stack and will switch it's polarity in an attempt to still deliver electrons. In a stack this leads to an effective blockage of the current thus limiting current flow through the entire stack of cells. Electrical engineering can help to overcome this issue by controlling the current that is allowed to pass through a single cell within a certain potential window (Andersen et al., 2013). BES up to 1 $m^3$ have been characterized but these reports are very scarce in literature (Dekker et al., 2009; Cusick et al., 2011) (Table 1.1).

## 2.5 Electrochemical measurements

Several electrochemical techniques are being used to study BES (Harnisch & Rabaey, 2012). A straight forward technique is to poise the potential of the electrode or cell voltage of the system and record the resulting current output, also known as potentiostatic control. When the potential is varied over time and the resulting current is recorded, this is called a potentiodynamic controlled experiment. Alternatively current can be set or varied to determine resulting potentials.

The internal resistances, or energy losses, of a fuel cell can be characterized using different methods (Figure 1.3 & 1.4). The four main methods that have been used in BES research are 1) current

interrupt, 2) calculation from a polarization or 3) a power curve and 4) electrochemical impedance spectroscopy (EIS) (Dominguez-Benetton et al., 2012). Interpretation of the results from all these measurements is a challenge since the biological nature of the catalyst, the polymorphous spacing of the catalyst on the electrode, the hydrodynamic patterns around the electrodes and accurate determination of substrate, product and biocatalyst concentrations can severely influence the outcome and interpretation of these measurements. A potentiostat (an electrical measurement device capable of accurately controlling and measuring potentials and currents) equipped with a 3 electrode setup and appropriate software is needed for polarization curves, EIS and current interrupt and (cyclic) voltammetry. A polarization curve can be recorded with rather simple means such as a variable resistor box.

**The current interrupt method** can be used to determine the ohmic resistance of a BES. A resistor is used to close the circuit which enables the cell to give a stable potential and current output. Subsequently the external resistor is removed and the instantaneous potential change ( in µs) is used for the calculation of the ohmic resistance by means of $R_{\Omega}=dV/I$ (Logan, 2008). Using electrode potentials in this equation gives an indication of the contribution of the individual electrode to the total ohmic resistance.

**A polarization and power curve** gives more insight into the various parts (Figure 1.3) of the total internal resistance of a BES (Logan et al., 2006; Liang et al., 2007; Clauwaert et al., 2008; Fan et al., 2008). By varying the cell potential, either by potentiodynamic control or by stepwise lowering of the external resistance, a polarization and power curve can be recorded (Figure 1.4). A polarization and power curve are a good method to (readily) verify the capabilities of the system under study, however this is an instantaneous measurement that can easily lead to overestimations.

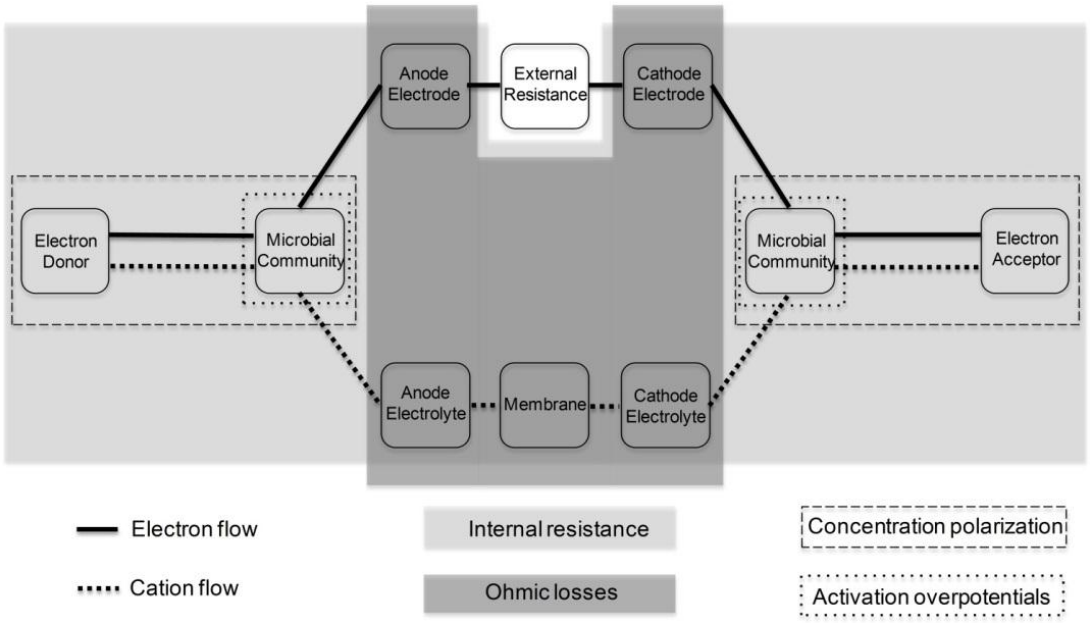



***Figure 1.3**: Schematic overview of internal resistances occurring in a BES. In an ideal situation, internal charge balancing is done by means of protons. However other cations but also anions can perform this task.*

The difference between the theoretical cell potential (calculated based on the redox reactions that occur at the electrodes and the Nernst equation) and the cell electromotive force $E_{emf}$ or open circuit voltage (OCV) occurs due to the enzymes or terminal electron transport molecules that are involved in establishing the transfer of electrons to or from the electrode. This is dependent on the metabolism of the microorganism(s) catalysing the reaction (Logan et al., 2006; Clauwaert et al., 2008).

Upon closing the circuit and driving a small current to run through the system, activation overpotentials are the largest source of voltage loss. Activation polarization losses can be determined by the Tafel equation when concentration polarization is not taken into account (Figure 1.3 and Figure 1.4, section A) (Logan et al., 2006; Freguia et al., 2007; Clauwaert et al., 2008).

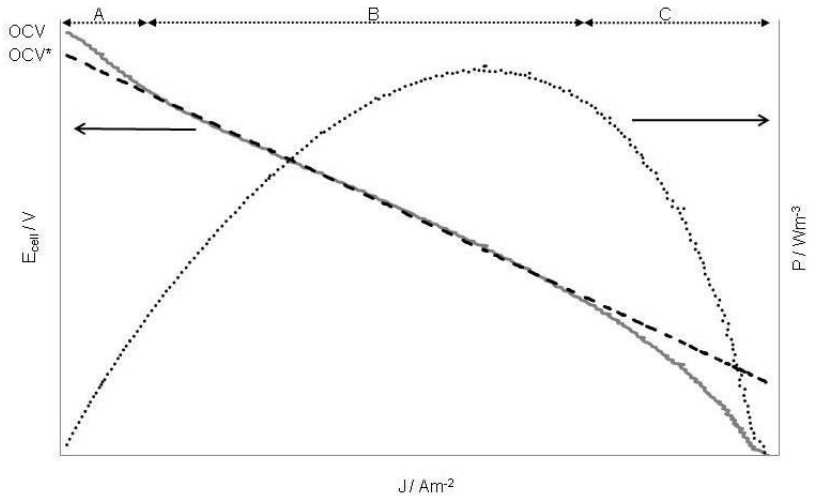



***Figure 1.4:*** *Typical polarization curve that can be recorded from a BES. OCV: open cell voltage OCV*: open cell voltage used to determine current dependent internal resistance. A: activation overpotentials are the dominant voltage loss in this region. B: current dependent voltage loss is the dominant voltage loss in this region. C: transport and concentration polarization are the dominant voltage losses in this region. ——— : Cell voltage ($E_{cell}$ / V ).....: Power density ( P / $Wm^{-3}$).* – – – *: Linearized Cell voltage to determine $R_{int}$.*

Allowing more current to flow through the system gives rise to ohmic losses (Figure 1.3 and Figure 1.4, section B). These losses are current dependent and become more influential compared to the overpotentials as more current is allowed to flow (Logan et al., 2006; Clauwaert et al., 2008). These losses are due to electrical contacts between electrode and current collector (Logan et al., 2006; Clauwaert et al., 2008), anolyte and catholyte conductivity, resistance to ion transport through the membrane/separator (Ter Heijne et al., 2006; Sleutels et al., 2009b) and the pH difference between both electrodes (Fan et al., 2008). A pH difference of 1 unit results in a voltage loss of 59 mV. Although the potential loss caused by the increasing pH difference is a thermodynamic parameter (i.e. it alters the theoretical cell potential), the change in pH is caused by an increase in current and non-perfect transport in the BES. The total sum of all internal losses during current generation can be determined from the linear part of the polarization curve with $R_{int}=(E_{OCV}^{*}-E_{cell})/I$ (Liang et al., 2007; Fan et al., 2008). To determine the influence of the different components in the total internal resistance, separate experiments need to be conducted such as conductivity, pH measurements and product and reactant concentrations. The voltage drop over the membrane can be measured with

reference electrodes on both sides of the membrane, corrected for conductivity and pH of the electrolyte (Ter Heijne et al., 2006; Sleutels et al., 2009b). The internal resistance calculated by ohm's law can also be expressed as a parameter of the reactor under specific operating conditions. This is done by replacing current (I) by current density (J). The resulting resistance is consequently expressed in $\Omega m^2$ or $\Omega m^3$.

Increasing the current even more leads to a non-linear voltage drop. The dominant factor for potential loss can now be related to mass transport limitation and concentration polarization (Figure 1.3 and Figure 1.4, section C). Examples are lack of reactant supply to the electrode or a build-up of product at the electrode. These voltage losses cannot be expressed as a resistance anymore due to their independence on the current. Mass transport and concentration polarization losses can be amended by adopting alternative cell designs or flow regimes (Sleutels et al., 2009a; Ter Heijne et al., 2010a).

The total internal resistance can also be obtained from a power curve from the notion that at maximum power the internal resistance is equal to the external resistance. Consequently the internal resistance can be calculated with $R_{int}=P/I^2$. Recording a polarization curve in a BES needs to be done with some care. Researchers have recorded curves with multiple fed-batch cycles and one external resistor per cycle, during one fed-batch cycle with multiple resistors and during continuous mode operation (Watson & Logan, 2011). Therefore it is advised that before comparing results, also experimental conditions are taken into account. This is exemplified by Winfield and co-workers who analysed the effect of scan rate, biofilm maturity and feedstock concentration on the polarization curve (Ieropoulos et al., 2010; Winfield et al., 2010).

**Electrochemical impedance spectroscopy** is a more sophisticated technique to characterize BES (He et al., 2006). It entails applying an alternating potential with set amplitude on a set cell potential. As opposed to current interrupt, polarization curves or voltammetry, this technique is non-invasive and can only give interpretable results when the system under study is in steady state. The results are analysed by fitting the data to an equivalent circuit with the dedicated software and internal resistance is determined from a Nyquist plot. However, the use of EIS has not been widely applied in BES research yet and therefore no consensus exists on frequency range, amplitude and interpretation of the data with the equivalent circuit (He et al., 2006; He et al., 2007; Logan et al., 2007; Manohar et al., 2008; Borole et al., 2010; Dominguez-Benetton et al., 2012)

**Cyclic or linear sweep voltammetry** (CV and LSV respectively) are electrochemical techniques used to study electron transfer mechanisms at the electrodes in more detail (Harnisch & Freguia, 2012). As with the techniques mentioned above, due to the microbiological nature of the catalyst, interpretation of results needs to be done carefully. Voltammetric techniques are used to characterize redox active species in a three-electrode setup. This characterization can be done on liquid samples, as such, centrifuged or filtered or on electrodes with the biofilm attached. By scanning all these fractions, one can get an idea of the nature (soluble, membrane bound, etc.) and the midpoint potentials of the redox active compounds present. These data can help to interpret the electron transfer mechanisms that are being employed at the specific electrode. As most liquids used in BES research are quite heterogeneous and sometimes undefined, midpeak potentials and current responses do not always coincide with theoretical patterns or patterns obtained from the pure compound in defined media. Examples of differences in physiological state of the microorganism and

a detailed discussion on the use of CV in BES research are clearly outlined by Fricke et al. (2008). Cyclic voltammograms are usually recorded under turnover (excess of substrate) and non-turnover conditions (lack of substrate). This allows determine maximum current and midpoint potentials of the redox system under study (Harnisch & Freguia, 2012). CV was successfully used to demonstrate the presence of various indigenously produced redox active mediators in the anolytes of various BES (Marsili et al., 2008; Pham et al., 2008b). Also biological catalysis of the cathodic oxygen reduction reaction was verified by CV (Cournet et al., 2010; Ter Heijne et al., 2010a).

**Surface-Enhanced Raman spectroscopy** in combination with CV or EIS allows for *in situ* investigation of electron transfer mechanisms at the molecular level in a biofilm without the need to disrupt the biofilm (Millo et al., 2011).

### 2.5.1 Reporting performance

Various different means of reporting power, current and potential have been witnessed in BES literature. It is however essential that outputs and results are reported in a standardized manner (Logan et al., 2006; Harnisch & Rabaey, 2012). In fuel cell research current and power are reported as density per electrode surface which is a good standard measure for chemical fuel cells. However, in BES research current densities have been reported in various different ways. From an environmental engineering perspective, $m^3$ are the preferred measure while from a (bio) electrochemical perspective $m^2$ electrode surface is the preferred measure. This has resulted in current and power output per $m^2$ of electrode surface, $m^2$ projected electrode surface, $m^2$ membrane area, $m^3$ total anode, cathode or reactor volume or $m^3$ net anode, cathode or reactor volume. One can easily appreciate that this way of reporting is prone to confusion. Therefore it is important to clearly provide data needed to convert between various measurements so studies by different authors can thoroughly compared and translated into the practical reality of environmental engineering.

## 2.6 Applications and proof of concepts

Applications for BES have tremendously widened over the last decade. To provide a framework for placing BES, three concepts are used (Figure 1.5). The first is defined as the extraction of the energy contained in a dilute (waste) liquid substrate into electrical energy (see §2.6.1). Due to various reasons but mainly the low power density BES are at this moment not yet competitive with current large scale energy producing or water treatment technologies. Secondly, besides only energy production, BES is also considered as a useful concept for product formation from waste streams (see §2.6.2). Thirdly, a BES is claimed to facilitate a reaction or control a microbial community in such a way that it provides a sustainable alternative for (non) existing technologies (see §2.6.3). A (non-exhaustive) literature review of representative labscale BES outputs is given in Table 1.1.

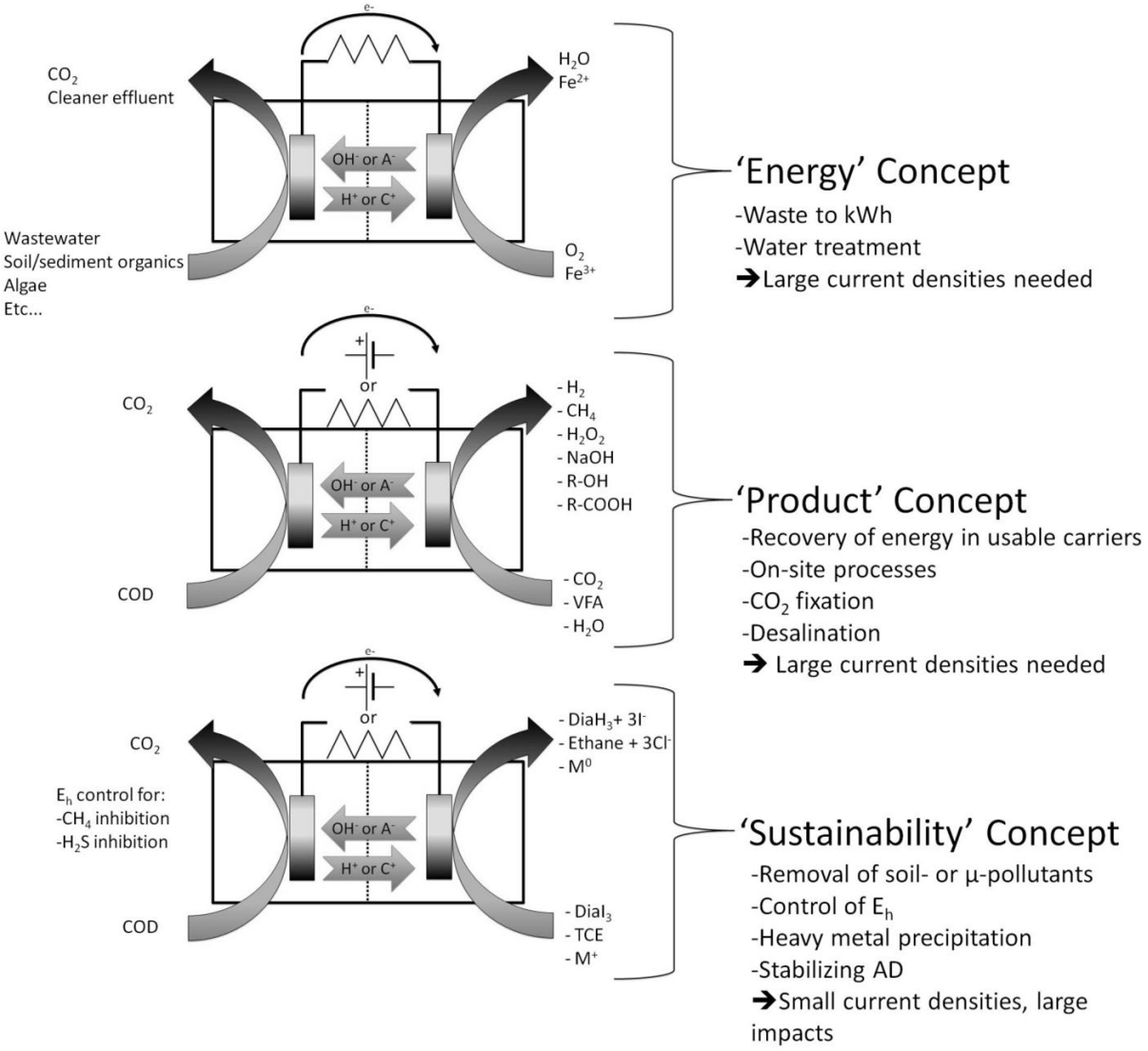



***Figure 1.5:*** *Three concepts for positioning a Bioelectrochemical system(BES). Some process can be placed in another concept than depicted here. For instance, metal precipitation can also be considered in the product concept. However, there it deals with large volumes and loading rates whereas in the sustainable concept heavy metals are removed in function of decontamination. The same accounts for remote sediment systems for sensor powering. These also fit in a sustainable concept as no expendable batteries and expensive exchange operations are needed. The algae in the product concept can be considered as effluent from a bioproduction process or they are the actual catalyst. For additional anode reactions see Figure 1.1.* $A^-$*: anion.* $C^+$*: cation.* $M^+$*: oxidized metal.* $M^0$*: zero valent metal.* $DiaI_3$*: diatrizoate, medical contrast medium.* $DiaH_3$ *: de-iodated medical contrast medium. TCE: trichloroethane.*

### 2.6.1 Energy & wastewater concept

Wastewater treatment

Wastewater has been regarded as a cost in all aspects. Due to its negative value and low organic material content, waste water is viewed as the preferred substrate for energy producing BES. Domestic wastewater is usually treated using different energy-demanding technologies with high

efficiency levels (Abegglen et al., 2008). Aelterman et al. (2006b) demonstrated that a MFC might occupy a market niche in terms of a stand-alone source of electricity for use in wastewater treatment. A maximum power density of 25 mW $m^{-2}$ was reached by oxidizing the organic matter from urban wastewater (Rodrigo et al., 2007). Puig et al. (2010) demonstrated the pH effect on electricity production at short- and long-term treatment of wastewater, achieving a power density up to 1.8 W $m^{-3}$ and an organic removal rate of 1.9 kg COD $m^{-3}$ $d^{-1}$ (77% COD removal efficiency). Ahn and Logan (2010) treated domestic wastewater at an organic loading rate of 54 kg COD $m^{-3}$ $d^{-1}$ (25.8% COD removal efficiency) and a maximum power generation of 0.42 W $m^{-2}$ (12.8 W $m^{-3}$).

However, wastewater also contains nitrogen which must be treated before discharge in the environment. Moreover, denitrification process (nitrate/nitrite reduction to nitrogen gas) is highly dependent on the influent carbon to nitrogen ratio. Nowadays this ratio is unbalanced limiting the removal efficiency. Autotrophic nitrate/nitrite removal in biocathode of BES has been successfully demonstrated by Clauwaert et al. (2007a; 2009) and Puig et al. (2011a). Autotrophic nitrogen removal in BES permits to denitrify at really low C/N ratios in comparison with conventional heterotrophic denitrification.

However, wastewater usually contains ammonium instead of nitrate which should be previously oxidised. Ammonium oxidation to nitrate could be done *in-situ* in a BES or in an external compartment. This concept was proven by coupling a double-chamber MFC with a nitrifying bioreactor (Virdis et al., 2008; Virdis et al., 2009) or by introducing a low dissolved oxygen concentration in the cathode (Virdis et al., 2010; Xie et al., 2011).

Industrial wastewaters can be highly contaminated with a wide range of organic and inorganic compounds, nutrients, chemical contaminants and heavy metals. The treatment of some of them has been assessed using BES. One of the best examples is brewery wastewater because of the type of organic matter and the lack of high concentrations of inhibitory substances (Feng et al., 2008; Wen et al., 2009). Brewery wastewater was treated in an air-cathode MFC with maximum power densities of 264 and 528 mW $m^{-2}$ respectively. Bakery, paper and dairy wastewater have been also evaluated in an air-cathode MFC for current production (Velasquez-Orta et al.). Paper wastewater was shown to produce the highest current density in comparison with the other influents, independent of substrate biodegradability. Heavily polluted wastewaters such as landfill effluents have been treated using a MFC. Electricity could be produced from those effluents with similar organic removal efficiencies to those obtained by a biological aerated filter under similar controlled conditions (Greenman et al., 2009). Landfill leachate treatment and electricity production in a MFC under high levels of nitrogen concentration (6.0 gN-$L^{-1}$) and conductivity (73.6 mS $cm^{-1}$) was also demonstrated (Puig et al., 2011b). Up to 8.5 kgCOD $m^{-3}$ $d^{-1}$ of biodegradable organic matter was removed at the same time as electricity (344 mW $m^{-3}$) was produced even at free ammonia concentrations around 900 mgN-$NH_3$ $L^{-1}$.

A MEC has also been applied to treat domestic and industrial (winery and swine) wastewaters. Swine wastewater with a COD of 12 - 17 g $L^{-1}$ was treated with COD removal efficiencies ranging from 19 ± 15 to 72 ± 4%, with hydrogen recoveries of 17 ± 7 to 28 ± 6% based on COD removal (Wagner et al., 2009). Organic matter removal efficiency and energy recovery were higher for MFC than MEC treating winery and domestic wastewaters. The energy recovery for winery wastewater in MFCs was 0.26 kWh $kg^{-1}$ COD with respect to -0.32 kWh $kg^{-1}$ COD for MECs. Hydrogen production costs of

winery and domestic wastewater with a MEC was determined as \$ 4.51 $kg^{-1}$ $H_2$ for winery wastewater and \$ 3.01 $kg^{-1}$ $H_2$ for domestic wastewater (Cusick et al., 2010) which is less than the estimated merchant value of hydrogen (\$6.00 $kg^{-1}$ $H_2$).

***Table 1.1.*** *Illustrative review of BES input, output performance and configurations.*

| | Application | Volume (mL) | Operational mode | Current (power) density | Other Output | Reference |
|---|---|---|---|---|---|---|
| Microbial Fuel Cells (MFC) | Domestic wastewater (organic matter) | 28 | Air-cathode MFC | 12.8 W $m^{-3}$ (0.42 W $m^{-2}$) | - | (Ahn & Logan, 2010) |
| | | 242 | | 1.8 W·$m^{-3}$ | - | (Puig et al., 2010) |
| | Domestic wastewater (nitrogen removal) | 716 | 2 chambered MFC | 8 W $m^{-3}$ (5 8A $m^{-3}$) | - | (Clauwaert et al., 2007a) |
| | | 250 | | - | - | (Virdis et al., 2009) |
| | | 650 | | 3.2 W $m^{-3}$ (20.8 A $m^{-3}$) | - | (Puig et al., 2011a) |
| | Landfill leachate | 900 | 2 chambered MFC | 0.002 mW $m^{-2}$ (0.003 A $m^{-2}$) | - | (Greenman et al., 2009) |
| | | 167 | Air-cathode MFC | 0.34 W $m^{-3}$ | - | (Puig et al., 2011b) |
| | Industrial wastewaters (bakery, brewery, paper and dairy) | 45 | Air-cathode MFC | 0.06 to 0.005 W $m^{-2}$ (0.12 to 0.01 A $m^{-2}$) | - | (Velasquez-Orta et al.) |
| | Refractory contaminants (Furfural) | 200 | 2 chambered MFC | 15.9 W $m^{-3}$ (39.9 A $m^{-3}$) | - | (Luo et al., 2011) |
| | Refractory contaminants (Ceftriaxone sodium) | 100 | Air-cathode MFC | 11 W $m^{-3}$ | - | (Wen et al., 2011) |
| | Refractory contaminants (Glucose and azo dye) | 220 | 2 chambered MFC | 0.4 W $m^{-2}$ (2.7 W $m^{-3}$) | - | (Li et al., 2010) |
| | Refractory contaminants (azo dye: methyl orange) | - | 2 chambered MFC | 0.006 W$m^{-2}$ | - | (Liu et al., 2011) |
| | Refractory contaminants (Nitrobenzene) | 364 | 2 chambered MFC | 17.06 W $m^{-3}$ | - | (Mu et al., 2010) |
| | Water desalination | 57 | 3 chambered microbial desalination cells | 31 W $m^{-3}$ (2 W $m^{-2}$) | 90% salt removal | (Cao et al., 2009; Mehanna et al., 2010b) |
| | Copper recovery | 800 | 2 chambered MFC with bipolar membrane | 0.8 W $m^{-2}$ (3.2 A $m^{-2}$) | - | (Ter Heijne et al., 2010b) |
| Plant/ Sediment MFC | Bioremediation of organic contaminants (acetate and benzoate) | - | Sediment batteries | 0.016 W $m^{-2}$ | - | (Bond et al., 2002) |
| | Bio-electricity and biomass production | - | Cylindrical plant MFC | 0.222 W $m^{-2}$ | - | (Helder et al., 2010) |
| | Solar energy powered MFC | 3300 | Solar energy powered MFC | 0.041 W $m^{-2}$ | - | (Strik et al., 2010) |
| | **Application** | **Volume** (mL) | **Operational mode** | **Input voltage** | **Output** | **Reference** |
| Microbial electrolysis Cells (MEC) | Winery and domestic wastewater | 28 | Two chambered MEC | - 0.90 V | 0.17 and 0.28 $m^3$ $H_2$ $m^{-3}$ $d^{-1}$ | (Cusick et al., 2010) |
| | Swine wastewater | 28 | Two chambered MEC | - 0.50 V | 0.9-1.0 $m^3$ $H_2$ $m^{-3}$ $d^{-1}$ | (Wagner et al., 2009) |
| | Water desalination | 57 | 3 chambered microbial desalination cells | - 0.55 V | 0.16 $m^3$ $H_2$ $m^{-3}$ $d^{-1}$ | (Mehanna et al., 2010a) |
| | Hydrogen peroxide generation | 518 | Two chambered MEC | - 0.50 V | 1.9±0.2 Kg $H_2O_2$ $m^{-3}$ $d^{-1}$ | (Rozendal et al., 2009) |
| | Caustic production | 3313 | Lamellar type reactor | -1.77 V (1015 A $m^{-3}$) | NaOH (3.4 wt%) | (Rabaey et al., 2010) |
| | Ethanol production | 1672 | Two chambered MEC | - 0.55 V | 1.82 mM EtOH; 0.012 $m^3$ $H_2$ $m^{-3}$ $d^{-1}$ | (Steinbusch et al., 2010) |

### 2.6.2 Product concept

Besides power production, researchers have looked for other opportunities to apply microbial catalysed electrochemical reactions. This has led to two paths of research. The first path makes use of the electrochemical properties of a BES and entails desalination of water in a three or more compartment BES (Cao et al., 2009) and the formation of inorganic products such as caustic caustic (Rabaey et al., 2010) or peroxide (Rozendal et al., 2009). The formation of these products is based on the better migration of certain cations compared to others. The second path is based on the bioelectrochemical properties of a BES. Here specialized microbial (mixed) cultures are supplied with a current which they can use to transform $CO_2$ into organic products or convert substrate organics into products, which is termed microbial electrosynthesis (MES). This will be further discussed in chapter 4 of this section.

Desalination

Current water desalination techniques (i.e. reverse osmosis, electrodialysis and distillation) are energy intensive (i.e. 3-5 kWh $m^{-3}$ for reverse osmosis) and some use membranes operated at high pressures (70 kPa) (Semiat, 2008). Water desalination can be accomplished in a three chambered MFC with two membranes (an anion exchange membrane placed adjacent to the anode, and a cation exchange membrane positioned next to the cathode). When current is produced by bacteria at the anode, ionic species in the middle chamber are transferred into the two electrode chambers, thereby desalinating the water in the middle chamber (Cao et al., 2009). This new method for desalination was able to remove 90% of the salt content without pressurizing or use of an external power source. The microbial desalination cell (MDC) design was optimized using oxygen at the cathode, rather than hexocyanoferrate, and combined with new ion exchange membranes (Mehanna et al., 2010b). In this way, 50% of the solution conductivity was reduced. However, the desalinated liquid needed a further polishing with reverse osmosis as the voltage was not stabile and the osmotic pressure was reduced between the anode and desalination chamber over time. To cope with this, the process was further optimized by operating the system as a microbial electrodialysis cell (MEDC). The energy efficiencies obtained ($\eta_E$) in the MEDCs reached 231 ± 59% (5 g $L^{-1}$ NaCl) and 213 ± 38% (20 g $L^{-1}$ NaCl), suggesting that sufficient hydrogen was produced to power the MEDC (Mehanna et al., 2010a).

Caustic soda and hydrogen peroxide production

Rabaey et al. produced caustic from brewery wastewater while producing currents up to 1015 A $m^{-3}$ (Rabaey et al., 2010). The lamellar type reactor with a total liquid volume of 1.63 L (66% anodic) produced caustic soda in the cathodic compartment taking advantage of the sodium diffusion from the anode and the proton consumption in the cathode compartments respectively. The energy input as electricity to caustic produced ratio was 1.6kWh $kg^{-1}$ NaOH which corresponds to an operational cost of around $ 0.1 $kg^{-1}$ low strength caustic. This price is lower in comparison to the market price of above $0.5 $kg^{-1}$ (Rabaey et al., 2010).

Hydrogen peroxide ($H_2O_2$) was produced in a MEC at an applied voltage of 0.5V (Rozendal et al., 2009). Cathodic reduction of oxygen to $H_2O_2$ was catalysed by inexpensive carbon materials. Under this condition, the MEC was capable of producing around 1.9 ± 0.2 kg $H_2O_2$ $m^{-3}$ $d^{-1}$ from acetate with energy requirements of around 0.9 kWh $kg^{-1}$ $H_2O_2$. However the low concentration of $H_2O_2$ produced (0.13 ± 0.01 wt%) is at this moment not suitable for sale. Both products (NaOH and $H_2O_2$) are

envisioned to be used for cleaning in place (CIP) as a waste stream is converted on-site into a cleaning solution.

### 2.6.3 Providing environmental services

Recalcitrant compound removal

Production of different types of products such as: dyes, explosives, pesticides, paper printing, colour photography, pharmaceutical, cosmetics and leather leads to streams of recalcitrant compounds that are not readily degradable under natural conditions and are typically not removed from wastewater by conventional wastewater treatment systems. Physico-chemical methods, such as adsorption, coagulation– flocculation and especially advanced oxidation (i.e. ozonation, photocatalysis, fenton oxidation,...) have been used to treat them. These processes are energy and cost intensive. BES technology can be considered as a reliable alternative to these processes. It has been demonstrated that microorganisms can degrade many types of toxic and refractory compounds, such as phenols, indoles, azo- dyes, halogenated compounds etc. and generate electricity simultaneously.

Furfural and acid orange are toxic and refractory pollutants, which are used in oil refineries and petrochemical refining units to extract dienes from other hydrocarbons. Luo et al. (Luo et al., 2011) demonstrated that electrons and protons were released from the biodegradation of furfural in the anode chamber and transported to the cathode chamber to drive the Fenton-like reaction catalysed by $FeVO_4$ toward degrading acid orange 7. Li and co-workers (Li et al., 2010) reported about the azo dyes removal mechanism in a double chambered MFC. The azo bond was biologically cleaved through azo dye decolorization under anaerobic conditions in the anode chamber and abiotically in the cathode chamber. The aromatic amines were removed in the aerobic chamber.

Nitrobenze originates from numerous industrial and agricultural activities, and can be abiotically removed at a cathode coupled to microbial oxidation of acetate at an anode as shown by Mu and co-workers (Mu et al., 2010). Finally, a model antibiotic (ceftriaxone) was shown to be oxidatively removed and boost current production from glucose in the anode of an air-cathode MFC (Wen et al., 2011).

The chlorinated solvent 1,2 dichloroethane (1,2 DCA) was removed in the anode of a MFC without production of any toxic intermediate at a maximum removal rate of 40 $gm^{-2}_{anode\ surface}d^{-1}$ (Pham et al., 2009). Biogenic palladium nanoparticles (bio-Pd) produced by the precipitation of Pd on the surface of bacteria have been used as a catalyst for the dehalogenation of trichloroethylene (TCE) and diatrizoate ($diaI_3$) in the cathode of a MEC (Hennebel et al., 2010).

Greenhouse gas-mitigation

Root residues (mainly organic compounds) from rice plants produce methane. It is estimated that worldwide rice agriculture contributes 7-20% of the total methane emissions (Sass et al., 2002; Denman et al., 2007). De Schamphelaire et al. (2008) showed that a Plant-MFC could offer the prospect to steer and control the sediment redox potential and thus abate undesirable processes such as methylation of metals and emissions of methane. Moreover the plant-MFC produced sustainable power up to 330 W $ha^{-1}$ from the oxidation of the plant-derived compounds. Mitigation of methane emissions from wetland has been hypothesized in various reports but never been studied thoroughly yet (De Schamphelaire et al., 2008; Kaku et al., 2008; Cabezas De Rosa, 2010;

Arends & Verstraete, 2012). Next to methane, nitrous oxide ($N_2O$) is another strong greenhouse gas. It has been shown that this gas can be removed in the cathode of a BES (Desloover et al., 2011). Greenhouse gas mitigation potential was identified as a potentially strong parameter in a life cycle analysis (LCA) of BES (Foley et al., 2010; Pant et al., 2011).

Heavy metal recovery/removal

Mining and metallurgical wastewaters and leachates contain heavy metals such as copper, nickel, cobalt, and zinc. A potentially new application of BES is the removal and recovery of metals. Mining and metallurgical industries are the main contributors of anthropogenic copper (Cu) emissions to the environment (Ter Heijne et al., 2010b). Ter Heijne and coworkers showed that $Cu^{2+}$, added as $CuCl_2$ at pH 3, could be reductively removed in the cathode compartment of an MFC with concomitant energy generation. Copper deposited under anaerobic and aerobic conditions on the graphite electrode. The authors proposed that $Cu^{2+}$ reduction as a cathodic reaction is an interesting option to consider for the improvement of MFC performance as the oxygen reduction reaction was also improved by the presence of copper.

Also uranium (U) and mercury (Hg) have been removed using BES. Gregory and Lovley (2005) demonstrated that *Geobacter sulfurreducens* can use electrons derived from electrodes to reduce $U^{4+}$ to the less soluble $U^{6+}$. A follow-up study showed that it may be possible to remove and recover uranium with poised electrodes (Williams et al., 2010). Power generation (433 mW $m^{-2}$) coupled to mercury removal (240 mg $Hg^{2+}$ $L^{-1}$ $d^{-1}$, 99% removal efficiency in batch) in the cathode of a MFC was shown to be possible. Mercury was recovered as elemental Hg (on the cathode surface) and $Hg_2Cl_2$ (deposited on the bottom of the chamber) (Wang et al., 2011).

Biosensors and environmental monitoring

The use of microbial techniques to monitor the natural environment was mainly done by means of toxicity assays. A vast array of research has already been done on enzymatic biosensors with glucose oxydase being the focal point (Kissinger, 2005). With the discovery of anode respiring bacteria researchers have seen the opportunity to create biosensors that directly link environmental parameters to a small current. Up till now, most research has been focussed towards biological oxygen demand (BOD) sensors (Di Lorenzo et al., 2009; Greenman et al., 2009). The applicability of a BES biosensor in the field remains to be proven. Several issues need to be addressed. These include calibration, functional stability of the active unit (i.e. biofilm or immobilized enzyme) under different operating and storage conditions and competitiveness with existing technologies (Kissinger, 2005). Small steps have been made towards understanding the behaviour of MFC-based biosensors by looking at toxicity responses (Patil et al., 2010a) and stable baseline outputs (Stein et al., 2010). Williams et al. used a BES sensor from a different perspective. Their approach was to monitor in-situ microbial activity in anoxic soils. They were able to link an increase in MFC current to an enhanced removal of $U^{4+}$ (Williams et al., 2010).

## 2.7 Benchmarks

To underpin the usefulness of a BES for a certain treatment or application, one has to consider the total cost and benefits, also in terms of sustainability, of applying the BES option compared to another option. Recently several researchers have laid the basis for an analysis of economic viability of implementing a BES system (Rozendal et al., 2008b; Fornero et al., 2010; Pant et al., 2011). A study on the LCA of high-rate anaerobic treatment respectively MFC and MEC by Foley et al. (2010) has revealed that a microbial fuel cell does not provide a significant environmental benefit to the conventional anaerobic treatment. Yet, a MEC can provide environmental benefits through the displacement of chemical products (e.g. NaOH, $H_2O_2$, ..). This study suggests that anaerobic digestion, which is furthermore empowered by recovery of e.g. phosphate or nitrogen (Carballa et al., 2009), is at present the more sustainable full scale technology and that the overall BES technology still has to strongly advance towards a target conversion of some 1000 A per $m^3$ reactor at $m^3$ scale in order to become competitive.The major drawback of LCA and economic analysis is that there is not a large pool of real-life data available, however several start-up companies are have been founded, so more knowledge and experience are expected in the near future.

### 2.7.1 BES relative to batteries and chemical fuel cells as a benchmark for energy density

To have a general benchmark for where the field of BES research stands in terms of power production, a comparison can be made with conventional energy carriers such as batteries and chemical fuel cells. In terms of biological substrate conversion rates and current generating potential, the best option is to compare a BES with anaerobic digestion (see 2.7.2). Normal household batteries, due to their chemical nature, can attain a very high power and current density up to 90 kW $m^{-3}$ (Table 2.1). This is mainly due to their close electrode spacing and highly conductive electrolytes. Chemical fuel cells have been developed to form a large group of varying reactor types. However in terms of configuration i.e. anode, separator and cathode, they are comparable to BES. CFC have a wide range of operating conditions in varying temperatures (– 25 $^0$C till +800 $^0$C), pressures and also substrates (various gasses and small alcohols). Current and power densities of CFC are usually reported per $m^2$ of electrode surface whereas these values in BES are reported per membrane, true electrode or projected electrode area. Current and power densities for BES are also reported per $m^3$ total or nett anode, cathode or reactor volume. This makes comparison with BES a bit more challenging but on the whole, 10-100 kW $m^{-3}$ (Table 2.1) is a reasonable estimate for CFC. Notwithstanding the advances that have been made in these technologies, a major drawback of chemical technologies is that they are not a sustainable technology yet. Batteries and CFC generally use primary, non-renewable energy sources. Indeed hydrogen and electricity for (re)charging are not yet readily available, thus need to be created from other sources (2 unit operations before current is obtained; hydrogen production followed by current generation). Whereas BES generally use waste streams that are most often readily available (1 unit operation; wastewater is directly converted into current). The electrode materials for CFC usually require noble metals which can be scarce, moreover, these materials are prone to fouling thus the fuels need to be processed before use. This can also be said of BES, especially considering oxygen reducing cathodes, but here biological alternatives are available. Last but not least, hydrogen gas used in CFC is difficult to store and transport. Yet, overall it is clear that conventional batteries and chemical fuel cells outrank by far the biological systems in terms of energy output per unit volume.

### 2.7.2. BES relative to anaerobic digestion as a benchmark in terms of conversion rates

The performance of BES in terms of economic viability has usually been compared to that of anaerobic digestion of (low strength) wastewaters. This is done because the same feeds (liquid biomass (waste) streams) are applicable for both systems and the same type of output (electrical energy) can be generated in both systems. The incoming biomass is first hydrolyzed, fermented and finally transformed by microorganisms into a final product (Verstraete et al., 1996; Angenent et al., 2004; Appels et al., 2008). The final product from an AD process is biogas ($CH_4$ and $CO_2$). The biogas is subsequently converted in a combined heat and power module (CHP). Biogas can also be converted into hydrogen gas by means of steam methane reforming. The heat obtained from the CHP is usually returned to the digester which is operated at mesophilic or thermophilic temperatures. The electrical power can be put to other use (Pham et al., 2006). In a BES, an electrical current is directly generated by the microbes at the anode electrode.

Two distinct differences between AD and BES can be observed. The first is that biomass processing by AD is perfectly capable of dealing with suspended and particulate organic materials. In BES however, particulate matter is difficult to process. Most systems nowadays are operated with three dimensional (3D) electrode materials, to support the current producing microorganisms, such as granules, felts and meshes of carbon, graphite and (or in combination with) noble metals (Logan et al., 2006; Logan et al., 2007; Aelterman et al., 2008a; Dumas et al., 2008a; Sleutels et al., 2009a). The use of these materials combined with suspended particles can cause clogging of the system which poses a serious threat towards sustained operation. The second difference is that using a BES, one has the option to produce direct electricity or various other energy carriers and products as discussed before.

During anaerobic digestion it is generally accepted that 1 kg COD can be converted to 4.16 kWh or 12.6 $*10^6$ C at a rate of 1 kg of COD per $m^3_{reactor}$ per hour (Pham et al., 2006). On average a yield of about 1 kWh of usable energy can be obtained in the form of electricity. The other 3 kWh are used for operating the mesophilic or thermophilic digester in an economic fashion or are lost during the conversion of the biogas to electricity (Pham et al., 2006). For BES to become competitive with anaerobic digestion as a means of waste water treatment, the rates of conversion of substrates consequently needs to be up to 1 kg of COD per $m^3_{anode}$ per hour. For a BES to be competitive with anaerobic digestion as a means of bio energy production a power density around 1 kW per $m^3_{anode}$ volume needs to be realized. For this work, the focus is on the anode, although we realize that the reaction in the cathode is most of the time the limiting factor to increase current production. Although it is a simplification to compare various, as yet still at the 1-10 L scale level, BES with $m^3$ scale anaerobic digesters, the comparison helps to assess the R & D priorities and the practical potentials of the respective systems. Therefore the main unit of comparison will be output per $m^3$ of reactor volume.

***Table 1.2**: Comparison of various (bio)-electricity producers with respect to energy density and conversion rates. Data are indicative and represent order of magnitude.*

| Size | Reaction | Electrolyte | Weight (g) | Volume (mL) | Operating time (h) | Operating potential (V) | Power density (kW $m^{-3}$) | Power density (W $Kg^{-1}$) | COD equivalent (kgCOD $m^{-3}$ $d^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Conventional batteries | $Zn/MnO_2$ | KOH | 24 | 8 | limited | 1,3 | 30 | 10 | 173[a)] | (Duracell)[b)] |
| | Li-ion | | 45 | 17 | limited | 3,6 | 90 | 35 | 520[a)] | (Panasonic) |
| Chemical fuel cells | H2 or reform gas /O2 or air | polymer membrane | (-) | (-) | continuous | (-) | 140 | 120 | 810[c)] | (Sundmacher, 2010) |
| Anaerobic digestion | COD to $kW_{el}$ & $kW_{heat}$ | (-) | (-) | 500-1000[d)] | continuous | (-) | 4 | (-) | 25 | (Pham et al., 2006) |
| BES anode | COD to $kW_{el}$ | (waste) water/ conductive membrane | (-) | 1-500 | continuous | 0,3-0,7 | 0,1 | (-) | 2,5 | This text |

(-) not applicable.

[a)] Based on the notion that 1 kg COD ~ 4.16 kWh.

[b)] Data from product specifications of the respective companies. Available online on the company website. Accessed December 2010.

[c)] This is an indicative sample for the Polymer Exchange Fuel Cell (Conte et al., 2009; Sundmacher, 2010).

[d)] AD reactor volume in $m^3$.

## 2.8 Substrate conversion rates

Although methanogenesis does not involve membrane bound electron transfer for all its electrons, all the electrons do have to pass through the membrane. This is either in the form of chemical oxygen demand as substrate and methane and carbon dioxide as products. Therefore, a true electron flux is present during methanogenic activity.

A default figure considered by engineers under stable operating conditions for substrate conversion rates is that 1 kg $VSS_{biomass}$ can convert 2 kg COD per day. Anaerobic digesters can be operated at organic loading rates of 5-25 kg COD per $m^3$ per day (Pham et al., 2006). These rates are obtained by suspended microorganisms, either fully in suspension or in granules. The electron donors and acceptors are also in the liquid phase, either in solution or in a particulate form. From these values a 'current' per square meter of biological surface area can be calculated. Considering that 1 kg $VSS_{biomass}$ contains approximately $10^{15}$ cells and an average cell diameter of 2 µm, it can be calculated that 1 kg $VSS_{biomass}$ contains 12.6 $*10^3$ $m^2$ biological outer membrane. Through this membrane 2 kg of COD are passing during 1 day. This results in a current density of 22.3 mA $m^{-2}$ biological membrane i.e. the total flux of electrons per $m^2$ biological membrane during removal of COD (Figure 1.7).

In the anode of a BES, electron transfer has to occur across the microbial cell membrane to the solid electron acceptor. Considering the same metabolic conversion rate as in AD i.e. 280 A kg $VSS^{-1}_{biomass}$ (2 kgCOD kg $VSS^{-1}_{biomass}$ $d^{-1}$) in a BES and a biologically relevant electrode area of 100 $m^2$ $m^{-3}$ with a biofilm thickness of 10 µm and density of 20 kg $VSS_{biomass}$ $m^{-3}$, a current of 0.056 A $m^{-2}$ electrode surface or 5.6 A/$m^3$ anode compartment can be reached (Figure 1.7) Cusick et al. (2011) actually reached a comparable output of 7.4 A $m^{-3}$ in the first pilot scale MEC. This calculated current density of 5.6 A $m^{-3}$ is 625 times smaller than the current density obtained in AD (i.e. 3500 A $m^{-3}$; Table 1.2). Several improvements to the above calculation can be made. Firstly, several researchers have observed anode biofilm thicknesses of 50-80 µm in experiments with low electrode surface to reactor volume ratios (Lee et al., 2009; Mclean et al., 2010; Nevin et al., 2010). It is not clear how such a thick biofilm can be supplied with sufficient substrates and drained in terms of metabolites. Especially proton transport from the biofilm to the bulk liquid is a limiting factor (Torres et al., 2008).

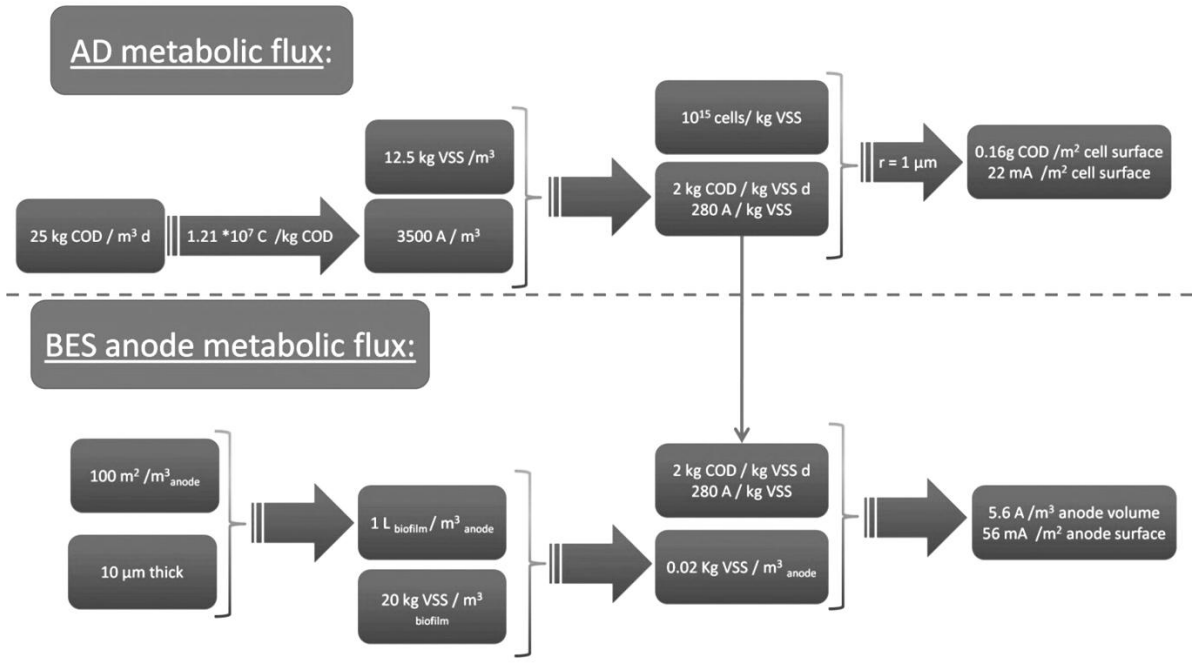



***Figure 1.7**: Calculation scheme for metabolic rates in AD and BES based on AD default values. This scheme is used to calculate the values in Table 4. C = Coulomb. VSS = volatile suspended solids. COD = chemical oxygen demand. r: radius. d: day.*

Secondly, higher metabolic conversion rates than assumed here have been reported, up till 22.3 kg COD $kgVSS^{-1}_{biomass}$ d (Lee et al., 2009). It stems to reason that an attached electrogenic microorganism can amp up its specific metabolic rate once it is effectively connected to an electrode. Thirdly, higher electrode surface areas per anode volume have been reported (see also paragraph 2.4.1), such as carbon or graphite felt with an actual surface area of $4.5*10^4$ -5.5 $*10^4$ $m^2$ $m^{-3}$ (Alfa-Aesar, London, UK and National Electrical Carbon Products BV., Hoorn, The Netherlands). For graphite granules surface areas of $10^4$ $m^2$ $m^{-3}$ (Freguia et al., 2008) up till $10^6$ $m^2$ $m^{-3}$ (Mersen, Wemmel, Belgium) can be found. These surface areas are determined through nitrogen absorption thus it remains to be seen which fraction of this surface is biologically relevant. For the following a surface area of 1000 $m^2$ $m^{-3}$ will be considered. As a fourth consideration, biofilm on an anode densities can vary, even up to 50 $kgVSS_{biomass}$ $m^{-3}$ (Lee et al., 2009). Taking all these considerations into account it can be seen that all these factors, when multiplied, are needed to reach a performance that comes close to AD when the aim is conversion of organic matter per $m^3$ of reactor volume. Comparing these data with Table 1.2 it can be seen that state of the art results are now up to 595 A $m^{-3}$ under sustained operation. This is only 17 % of the maximum AD metabolic rate obtained and was achieved in a L-scale BES reactor.

## 3. The plant microbial fuel cell

Photosynthesis by microorganisms and algae as a driving force for a BES has been researched in both the anode and the cathode. Recently several reviews of BES based on photosynthesis were presented (Rosenbaum et al., 2010b; Rosenbaum & Schröder, 2010; Strik et al., 2011). In the anode various strategies have been explored to convert sunlight into oxidizable compounds or into direct electricity by means of microorganisms(Pisciotta et al., 2011). In the cathode options for photo driven reactions are limited. The most viable option is to use algae for *in-situ* generation of oxygen to drive the cathodic reduction reaction. Strik et al. (2010) have developed a bio-electrode with reversible

properties i.e. during illumination it produced oxygen and acted as a cathode while during dark periods the electrode acted as an anode.

A niche in the energy concept is occupied by microbial fuel cells in natural environments. Sediment MFCs have been described by the pioneering research of Reimers, Tender and Bond (Reimers et al., 2001; Bond et al., 2002; Tender et al., 2002). These types of microbial fuel cells are the only designs that have shown to have a commercial application. These benthic systems are used to power remote sensors that require long-term remote low power supplies (Donovan et al., 2008; Tender et al., 2008; Dewan et al., 2010). The main advantage of these systems is that on-site battery replacements are no longer required. Dewan et al. (2010) made a step forward by developing a method to evaluate and optimize energy harvesting when a MFC was used with a capacitor to power sensors monitoring the environment.

One of the limiting factors to high power output of a sediment MFC is the low flux of reduced compounds toward the anode electrode. These are not actively provided since transport relies on passive mechanisms like natural water currents and settling of (dead) organic material. A solution to this problem was found by adding living, photosynthesizing plants to the system. A living plant excretes a range of oxidizable organic material from its roots by active processes (exudation, excretion) or via passive processes (dead root cells, lysates, sloughing off of cell material due to growth) which can amount up to 40% of the plants' photosynthetic productivity, and thus be a continues supplier of fuel for a sediment system (Neumann & Römheld, 2007; Strik et al., 2008). The plant powered sediment MFC concept was proven by three independent research groups in 2008 (De Schamphelaire et al., 2008; Kaku et al., 2008; Strik et al., 2008). Since then the research on this topic has expanded to include plants in more engineered systems (Helder et al., 2010), development of specific reactor designs to minimize internal resistance and development of a plant growth medium to minimize alternative electron acceptors in the anode zone. Grass species such as rice (*Oryza sativa*, various cultivars) have mostly been used for Plant-MFC research (De Schamphelaire et al., 2008; Kaku et al., 2008; Takanezawa et al., 2010; Chiranjeevi et al., 2012) but also *Glyceria maxima* (Strik et al., 2008) *Spartina anglica*, *Arundinella anomala*, *Arundo donax* (Helder et al., 2010), *Lemna minor* (Hubenova & Mitov, 2012) and *Echinochloa glabrescens* (Bombelli et al., 2013) have shown to be useful for electrical power generation.

The plant-powered microbial fuel cell has a range of potential benefits compared with other biobased energy harvesting technologies. For instance there is no competition for arable land, moreover, wetland rice cultivation has been identified as one of the possible options to install the Plant-MFC (Helder et al., 2013a). The Plant-MFC has also been envisioned to be implemented in greenhouse horticulture systems. This means that the food vs. fuel debate, which is occurring for other bioenergy schemes such as corn-ethanol or biodiesel, is no issue for the plant–MFC technology. Another beneficial aspect is limited $CO_2$ release during the process of energy conversion as any released $CO_2$ can be taken up by the plant during photosynthesis. When a plant-MFC is implemented in urban areas they can be used in a green roof configuration, acting as a power producer with passive benefits such as increased water retention, isolation of the building and providing environmental niches in an urban environment (Helder et al., 2013a).

## 4. Microbial electrosynthesis

Microbial electrosynthesis (MES) is the process of "microbially catalysed synthesis of chemical compounds in an electrochemical cell" according to Rabaey et al. (Rabaey & Rozendal, 2010). Thus MES can feed microorganism electrons to reduce $CO_2$ or substrate organics into other organic products in a cathode. It also enables balancing or redirecting oxidation reactions in an anode by removing excess electrons. Although the term was only coined in 2010 (Nevin et al., 2010), the process has been in development since 1999 when Park et al. (1999) showed that microorganisms were able to reduce fumarate to succinate and $CO_2$ to methane by feeding them an electrical current. However a mediator was needed to accomplish the process.

Direct feeding of electrons without the use of a mediator was shown by the formation of organic acids. The cathode compartment of a BES was inoculated with a homoacetogenic culture in order to reduce carbon dioxide to acetate and other multicarbon extracellular products (i.e. 2-oxobutyrate). The electron recovery in acetate and 2-oxobutyrate was 86% of the electrons transferred at the cathodes. Subsequently a range of other microorganisms were investigated for their ability of biofilm based electrochemical $CO_2$ reduction to organic carbon. Most homoacetogens showed the ability to indeed directly accept electrons from an electrode for $CO_2$ reduction. Interestingly *Acetobacterium woodii* was not able to perform this conversion. This was attributed to the fact that *A. woodii* has a sodium based ATPase instead of a proton based ATPase as all other tested homoacetogens had (Nevin et al., 2011b). The authors suggested that although acetate has economic value, a more important consideration is that acetate is formed from acetyl coenzyme A (acetyl-CoA), which is the central intermediate for the genetically engineered production of a wide range of chemical commodities as well as potential liquid transportation fuels (Nevin et al., 2010). To achieve the production of various other compounds, genetic tools were developed and optimized to use *Clostridium ljungdahlii* as a model microorganism for genetic engineering of homoacetogens (Leang et al., 2012).

Previous studies aimed at establishing a biofilm on the cathode. For that reason carbon electrodes were used and cathode potentials were set to levels that did not allow for any significant $H_2$ formation. To optimize the use of carbon electrodes several modifications were done which showed that adding carbon nanotubes or metal catalysts markedly improved (3-7 fold) electrosynthesis rates (Zhang et al., 2013). Stainless steel plates have been uses as electrodes to drive the reduction of fumarate to succinate with a pure culture of *Geobacter sulfurreducens* (Soussan et al., 2013).

Steinbusch et al. (2010)reported on the biological reduction of acetate with hydrogen to ethanol. To stimulate acetate reduction at the cathode with mixed microbial cultures they applied a cathode potential of -0.55 V. In total, four major products were formed at the cathode: ethanol, hydrogen, n-butyrate, and the non-reversible reduced methyl viologen. Ethanol production had a coulombic efficiency of 49%. The highest production rate of ethanol production was 60 mg $m^{-3}$ cathodic compartment $d^{-1}$. As a follow-up study, chain elongation of acetate was achieved up to caproate and caprylate by means of *in-situ* produced $H_2$ (Van Eerten-Jansen et al., 2013).

## 5. Objectives, rationale and outline of this work

The plant microbial fuel cell has been described previously in 2008 by three independent groups (De Schamphelaire et al., 2008; Kaku et al., 2008; Strik et al., 2008) however, there were and are still many questions to be answered regarding this technology. Within the framework of the EU-FP7 project 'PlantPower', which was a collaborative effort between 3 universities, 2 research institutes and 4 companies, the focus of this work was on a plant-MFC in sediment environments. This work is aimed at clarifying opportunities where the plant-MFC technology can help to solve the four challenges that mankind is facing as mentioned in the first section of the introduction.

To optimize the power output of the sediment Plant-MFC it is of importance that the anode interacts well with the plant roots where rhizodeposition takes place and has a reliable electrical connection to the current collector. In **Chapter 2**, placement of the anode and several anode materials were investigated for their suitability in a sediment Plant-MFC.

It has been hypothesized that placing an anode in a reduced environment can direct the metabolic flux away from methane production (a potent greenhouse gas) by means of generating an electrical current. In **Chapter 3** the results of a study are presented that diversify the possible application of a sediment Plant MFC beyond electrical power harvesting.

The cathode remains often a limiting factor in electrical power harvesting by means of microbial fuel cells, this holds also true for sediment Plant-MFCs. In **Chapter 4**, an onset is made to understand improvements regarding the internal resistance of a sediment Plant-MFC and how the cathode configuration can help to optimize this.

Low power output of sediment-Plant MFCs can hamper the technology to be economically viable. Therefore other, more valuable product outcomes need to be found. In **Chapter 5** the feasibility of integrating a constructed wetland for wastewater treatment with a BES for peroxide production and additional disinfection are presented.

Up till now only low value products have been created by the reducing equivalents generated at the anode i.e. electrical power, oxygen reduction to water and/or hydrogen peroxide and clean water. The current generated at the anode can also be used to make high value product outcomes. In **Chapter 6**, the focus is on generating high value products from an electrical current by means of MES.

In **Chapter 7**, a general discussion on the combined work, together with some future perspectives for further research is presented.

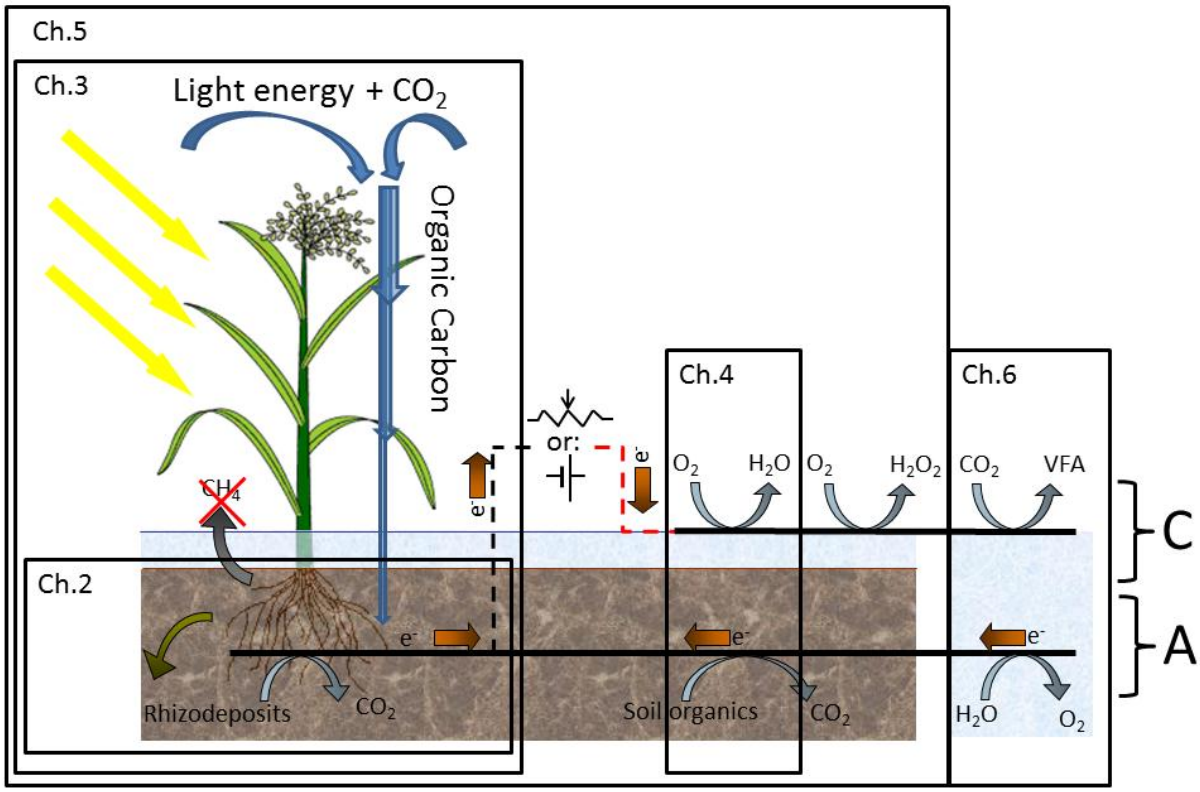



***Figure 1.8**: Schematic overview of the links between the five research chapters. Ch.: chapter. VFA: volatile fatty acid. C: Cathode processes. A: Anode processes.*  *: variable resistor indicating the power harvesting (MFC) option.*  *: power supply indicating the power investment option (MEC or MES).*

# CHAPTER 2: GRANULAR CARBON AS ANODE MATERIAL FOR (PLANT) SEDIMENT MICROBIAL FUEL CELLS

This chapter has been redrafted after:

Arends JBA, Blondeel E, Tennison S, Boon N, & Verstraete W (2012)
Suitability of granular carbon as an anode material for sediment microbial fuel cells.
J*ournal of Soils Sediments* 12(7):1197-1206.

## Abstract

Sediment-microbial fuel cells (SMFC) are bio-electrochemical devices that are able to oxidize organic matter directly into harvestable electrical power. The flux of organic matter into the sediment is rather low, therefore other researchers have introduced plants for a continuous supply of organic matter to the anode electrode. Until now only interconnected materials have been considered as anode materials in SMFC. Here granular carbon materials were investigated for their suitability as anode material in sediment microbial fuel cells. Laboratory microcosms with 8 different electrode materials (granules, felts and cloths) were examined with controlled organic matter addition under brackish conditions. Current density, organic matter removal and microbial community composition were monitored using 16S-rRNA gene PCR followed by Denaturing Gradient Gel Electrophoresis (DGGE). The main parameters investigated were the influence of the amount of electrode material applied to the sediment, the size of the granular material and the electrode configuration. Felt material had an overall superior performance in terms of current density per amount of applied electrode material i.e. felt and granular anode obtained similar current densities (approx. 50–60 mA $m^{-2}$) but felt materials required 29% less material to be applied. Yet, when growing plants, granular carbon is more suited because it is considered to restore, upon disturbance, the electrical connectivity within the anode compartment. Small granules (0.25–0.5 mm) gave the highest current density compared to larger granules (1-5 mm) of the same material. Granules with a rough surface had a better performance compared to smooth granules of the same size. The different granular materials lead to a selection of distinct microbial communities for each material, as shown by DGGE. Granular carbon is suited as anode material for sediment microbial fuel cells. This opens the perspective for application of MFC in cultivated areas. In a wider context, the application of granular carbon electrodes can also be an option for *in-situ* bioremediation of contaminated soils.

## 1. Introduction

Microorganisms are able to reduce solid materials (mostly metal-(hydr)oxides) while oxidizing organic carbon in the soil. This phenomenon of microbial respiration has successfully been applied to construct microbial fuel cells (MFC) (Logan et al., 2006). These devices consist of an anaerobic compartment where microbes are able to oxidize organic matter while delivering electrons to a solid electrode. These electrons are passed through an external circuit and end up in the cathode compartment in a final reduction reaction. The compartments are physically separated by means of an ion exchange membrane to ensure electro neutrality and prevent the crossover of reactants. These reactor configurations enable direct electrical power recovery from a wide range of liquid streams i.e. wastewater (Aelterman et al., 2006b). Power densities obtained in these types of reactor systems reach values of 10–100 W $m^{-3}$ (Aelterman et al., 2006b). Current densities range from as low as 4 mA $m^{-2}$ to 2 A $m^{-2}$ (Pant et al., 2010a). The MFC has been successfully applied to waterlogged sediment systems. Here the anode is placed in the anaerobic sediment layer, the (biological) oxygen reducing cathode is floating in the water above. The membrane is not installed but its function is maintained by the sediment layer on top of the anode. This easy to maintain system has been developed in marine settings (Reimers et al., 2001) but later freshwater systems were also tested (Holmes et al., 2004; Mohan et al., 2009). The sediment MFC (SMFC) is the first MFC design that has been reported with a useful application, mainly powering different types of remote sensors (meteorological buoy etc. (Tender et al., 2008)). Typical peak power outputs obtained with unmodified anodes are 0.2–55 mW $m^{-2}$ (Scott et al., 2008b) whereas current densities can amount to 100 mA $m^{-2}$ before mass transport becomes limiting (Scott et al., 2008b). Note that waterlogged soils are also applicable for anode placement. During this text, the word 'sediment' will be used to indicate both.

Applications were gradually expanded from marine environments into brackish and fresh environments, however the flux of organic matter towards the anode still remains rather low. A major portion, 20–60%, of the net fixed organic carbon during photosynthesis can be deposited in the soil as rhizodeposits (dead root biomass, active secretions or diffusive leakage) (Neumann & Römheld, 2007). Therefore a planted sediment fuel cell (PMFC) has the potential to provide long term sustained power output due the continuous photosynthetic activity of the plant. Indeed, several research groups found that adding plants to sediment fuel cells resulted in more power and current output. Rice plants (*Oryza sativa)* were used in sediment type fuel cells (De Schamphelaire et al., 2008; Kaku et al., 2008; Domínguez-Garay et al., 2013) while reed mannagras (*Glyceria maxima*) and common cordgrass (*Spartina anglica*) were used in a reactor type system containing a membrane and a separate cathode (Strik et al., 2008; Timmers et al., 2010). Comparing planted with non-planted SMFC, De Schamphelaire et al. (2008) found a 7–9 fold increase in power density and a 3 fold increase in current density of the planted systems vs. the non-planted systems amounting to 33 mW $m^{-2}$ sustained power production.

Until now the PMFC has mostly been explored in reactor type settings with the anode compartment completely filled with electrode material, either cloth or granular carbon (De Schamphelaire et al., 2008; Strik et al., 2008; Helder et al., 2010). SMFC have only been researched with solid connected materials such as carbon or stainless steel felts, cloths, rods etc. (Reimers et al., 2001; Dumas et al., 2008a; Scott et al., 2008b; Tender et al., 2008; Jian-Hai et al., 2009). In order to make a planted MFC widely available, for instance in greenhouses or (cultivated) wetlands, there is a need for granular

material that can readily interact with the roots but is not irreversibly damaged when agricultural practices are applied. One should be able to plant or harvest in such matrices. Granular materials are easily mixed with sediments and are able to create new connections after disturbance of the packed bed. Felt or cloth materials can be easily damaged but are not readily reconnected. Solid materials are not easily damaged but do not provide a large surface area for interaction with the plant and the electro-active microorganisms and are also hampering agricultural practices. In this study a range of carbon materials have been tested in microcosms to investigate the suitability of the use of granular materials as anode material for P- and SMFC. Parameters such as 1) amount of electrode material, 2) electrode configuration (loose or interconnected) and 3) granule size have been taken into account to compare performance between various materials.

## 2. Materials and methods

### 2.1 Experimental setup

Glass jars with a volume of 350 mL were used for these studies (Figure 2.1). Various anode materials were mixed with a layer of sand (Table 2.1). Materials were used as supplied, except for M1a which was washed with tap water to remove the dust. Cloth and felt materials had a projected area of 4*4 $cm^2$ and 1 layer, interwoven with a graphite rod (5 mm diameter; Morgan, Belgium) as current collector, was placed in the anode compartment. Granular material was mixed in a 1:1 volume ratio with sand (70.5 w-% = 0.25–0.5 mm diameter, pH = 9.9 in 1:10 dilution). A graphite rod was introduced in the middle of the sand/granule layer, at the same position as the cloth materials. In this text, the AR-value refers to the percentage of volume of the anode compartment which is occupied by the electrode material, e.g. a ratio of 1:1 corresponds to AR-50. To accommodate different percentages of felt material, more layers were added to the current collector. The same volume of sand was placed on top of the anode, which can be considered a membrane, followed by an equivalent volume of liquid. M9 medium modified with 13 g $L^{-1}$ NaCl and 14.7 mg $L^{-1}$ $CaCl_2$ was used in all tests (Rabaey et al., 2005a) therefore the microcosms can be considered brackish at pH 8, ensuring a good conductivity and no pH inhibition of the anode (Logan et al., 2006). In this work, no plants were used, because varying rhizodeposition patterns and plant/microbe interactions might obscure outcomes directly related to the materials under study. Organic carbon was instead provided as 20 mM of COD (chemical oxygen demand), sodium acetate, in the liquid of the anode compartment at the start of the experiment. This corresponds to about 2.5 ton dry matter $ha^{-1}$ on the assumption that 70 % of organic dry matter is COD. For the long term test 100 mM COD (12.5 ton dry matter $ha^{-1}$) was added (1:1 mix of sodium acetate and starch). To maintain current productivity, COD additions in the form of a concentrated stock solution of COD in M9 were injected at random places with a syringe, when current dropped. To provide electroactive microorganisms and thus ensure a fast start-up of the SMFC, 5 vol-% effluent of an acetate oxidizing anode (operated in the lab for over a year) was added to the anode compartment at the start of the incubation (Clauwaert et al. 2007b). Cathodes were 3 * 4 $cm^2$ carbon felts (Table 2.1; #4) incubated in the recirculation liquid of an oxygen reducing biocathode operated in the lab for over a year (Clauwaert et al., 2007b). To counteract evaporation, cathodes were replenished with distilled water when needed. All incubations were conducted in duplicate at room temperature 22 ± 2°C.

***Table 2.1:*** *Anode materials used in this study.*

| **Material code** | **M1a** | **M1b** | **M2** | **M3** | **M4** | **M5** | **M6** | **M7** | **M8** |
|---|---|---|---|---|---|---|---|---|---|
| **Supplier** | Mersen | Mersen | MAST | MAST | Alfa Aesar | MAST | MAST | MAST | MAST |
| | | | | | **Properties** | | | | |
| **Form** | Granules | Granules | Beads[a] | Beads[a] | Felt | Felt | Felt | Cloth | Cloth |
| **Precursor** | Phenolic resin and polymers | M1a | Phenolic resin | Phenolic resin | Resin | Viscose fiber | Viscose fiber | Viscose fiber | Viscose fiber |
| **Size (mm)** | | | **Diameter** | | | **Thickness** | | **Fiber diameter** | |
| | 1-5 | 0.25-0.5 | 0.25-0.5 | 0.25- 0.355 | 3.18 | 1.5 | 2.5 | 0.01-0.005 | 0.01-0.005 |
| **Density** | | | **kg $L^{-1}$** | | | **g $m^{-2}$** | | **g $m^{-2}$** | |
| | 1.8[b] | 0.8 | 2.6 | 0.5 | 240 | 118 | - | 180 | 100 |
| **BET surface area ($m^2 g^{-1}$)** | 0.5-1.3 | - | 1374 | 553 | 2 | 1231 | 365 | 900-1100 | 900-1100 |
| **Elemental composition** | | | | | | | | | |
| C Atom-% | 96.97 | - | 97.2 | 97.5 | >99 % | - | - | - | - |
| O Atom-% | 3.03 | - | 1.8 | 1.7 | - | - | - | - | - |
| N Atom-% | / | - | 1 | 0.8 | - | - | - | - | - |
| **Potential of zero charge (pH)** | - | - | >10 | >10 | - | >10 | >10 | >10 | >10 |
| **Remark** | Amorphous remainder of electro graphite production | Ground M1a granules | Smooth porous bead, highly mesoporous core, 38-40 % activated to create a high microporosity. | Smooth porous bead, highly mesoporous core, not activated, therefore has limited microporosity | - | ~30% Activated | carbonized | Knitted ~30% Activated | Knitted ~30% Activated |

Data as supplied by the different companies in personal communication or in publically available product data sheets. Mersen; Wemmel, Belgium. Alfa Aesar; Karlsruhe, Germany. BET: Brunauer-Emmet-Teller.

[a]: beads possess identical chemistry but a lower porosity as described in Tripisciano et al. (2011).

[b]: Determined by Clauwaert et al.(2007a).

/ : not detected.

- : not available.

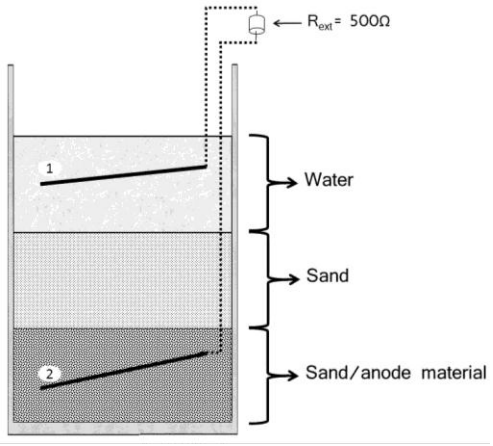



***Figure 2.1*** *Schematic of the sediment microcosms used for testing of various anode materials. 1) Graphite rod connected to graphite felt acting as a cathode. 2) Graphite rod current collector connecting the anode materials under study to the 500 Ω resistor. Each layer was approximately 2 cm high. "Water" indicates the standing water level, however the complete setup was saturated so no active flow from cathode to anode could occur. Electrical connection:* ····· .

## 2.2 Electrochemical monitoring & chemical analysis

Cell potential over a 500 Ω resistor and anode or cathode potential vs. an Ag/AgCl reference electrode (BASi inc. United Kingdom) were measured continuously (HP4907A, Agilent benchlink datalogger). The reference electrodes were regularly monitored versus a calomel electrode (+244 mV vs. Standard Hydrogen Electrode (SHE); QIS, the Netherlands). Polarization curves were recorded with a potentiostat (Bistat, Biologic, France) at a scanrate of 1 mV $s^{-1}$ following a 20 min. stabilization period in open circuit. Electrochemical calculations were performed according to Logan et al. (2006). Current and power density are reported normalized to the total surface area of the microcosm (0.0034 $m^2$).

Alternative electron acceptors $NO_2^-$, $NO_3^-$ and $SO_4^{2-}$ were determined by ion chromatography as previously described (Clauwaert et al., 2007a). Chemical oxygen demand was determined by means of a standard kit according to the manufacturers procedures (Macherey-Nagel, Germany). Samples from the anode compartment were taken at random places.

## 2.3 Microbial community & material structure analysis

Samples from the sand and sand mixed with M1a, M1b and M2 were taken at the end of the long term incubation (55 days) for analysis of the bacterial community structure (1 mL sediment and 1 mL pore liquid) and for Scanning Electron Microscopy (SEM) (1 mL sediment). Samples were taken from at least 5 random positions per microcosm in order to obtain a homogenous representative sample. Total DNA was extracted (Boon et al., 2000) followed by amplifying the bacterial 16S rRNA gene with primers 338F-GC and 518R. The different amplicons were separated using Denaturing Gradient Gel Electrophoresis (Carballa et al., 2011). Obtained profiles were clustered using BioNumerics software v5.1 (Applied Maths, Belgium). Theoretical ecological parameters Range weighted richness (Rr; a measure of habitability of an environment) and Community organization (Co; an approximate of functional diversity) were attributed according to the microbial resource management (MRM) toolbox (Read et al., 2011). Samples for SEM were immediately fixed by subsequent incubation in 2.5

% glutaraldehyde (2 hr) and 1% osmium tetroxide (1 hr) with 3 times washing with 0.1 M phosphate buffer (pH 7.5) in between. Subsequent dehydration was done with 30, 50, 70, 90 and 3 * 100% ethanol (20 min per step) with finally overnight drying in a desiccator.

# 3. Results

## 3.1 Initial material screening

Eight different materials (Table 2.1) were screened for their ability to receive electrons from an electro active microbial community in a sediment setting for about 10 days. From this initial screening, the big granules with a diameter of 1–5 mm (M1a), gave the best performance (90 coulombs (C) transferred), followed by all the felt/cloth materials (M4-M8) which gave about the same number of total coulombs transferred for all different materials (20–40 C). The least performing materials were the small granular carbon materials with a diameter < 0.5 mm (M2 & M3) (7-11 C) (Figure 2.2).

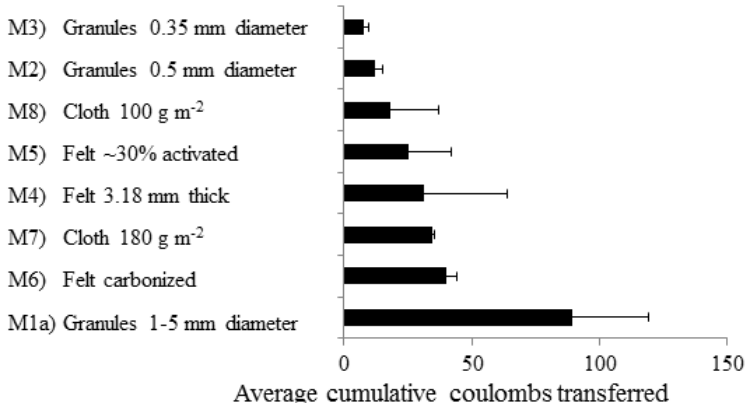



***Figure 2.2*** *Initial screening of various materials showed that bigger granules (1-5 mm) transferred more coulombs followed by felt/cloth materials and smaller granules (0.25-0.50 mm). The error bars indicate standard deviation (n=2).*

## 3.2 Influence of particle size

The initial experiment indicated that particle size had a large influence on electron transfer capabilities within the anode. It was hypothesized that a certain size distribution in relation with the sand is optimal for electrical connectivity in the soil or sediment. To investigate this, the large granules (M1a) were grinded to smaller particles. From this ground material the particles in the range of 0.25–0.50 mm were selected (M1b). Both materials (M1b & M2) had the same size as the major fraction of the sand. At an AR-50, the ground particles were compared to the original particles of 0.25–0.50 mm (M2), the large 1-5 mm particles (M1a) and a graphite rod only. After three weeks of incubation, electrical current densities averaged 55.2 ± 11.7mA $m^{-2}$ with a maximum of 77.7 mA $m^{-2}$ for the ground granules (M1b). The 1–5 mm granules (M1a) reached an average current density of 25.2 ± 7.74 mA $m^{-2}$ with a maximum of 37.9 mA $m^{-2}$. The original small granules (M2) averaged 5.33 ± 7.9 mA $m^{-2}$ with a maximum of 35.4 mA $m^{-2}$ (Figure 2.3a). The microcosm with only the rod as the current collector averaged 1.01 ± 0.37 mA $m^{-2}$ with a maximum of 1.87 mA $m^{-2}$. The results of the sustainable current production were corroborated by the maximum current densities obtained during the forward sweep of a polarization curve; 140.1 ± 42.4 mA $m^{-2}$ 38.8 ± 10.4 mA $m^{-2}$ and 8.0 ± 6.4 mA $m^{-2}$ were the average maximum current densities obtained during this time period for M1b,

M1a and M2 respectively. As current densities are the result of both anodic and cathodic processes, an examination of the anode potential is needed.

The minimum anode potentials, as determined from the open circuit potential from the polarization curves, indicated that all materials (rod, M1a, M1b) except for M2 were able to reach a low anode potential, i.e. - 0.067 till - 0.185 V vs. SHE at day 18. The anode potential of M2 varied around + 0.2 V vs. SHE (Figure 2.4b). The low anode potentials found here, correspond to an anoxic environment with COD oxidation as normally found in anodes of MFC (Logan et al., 2006). The higher anode potential corresponds with the low current density as indicated earlier (Figure 2.3a).

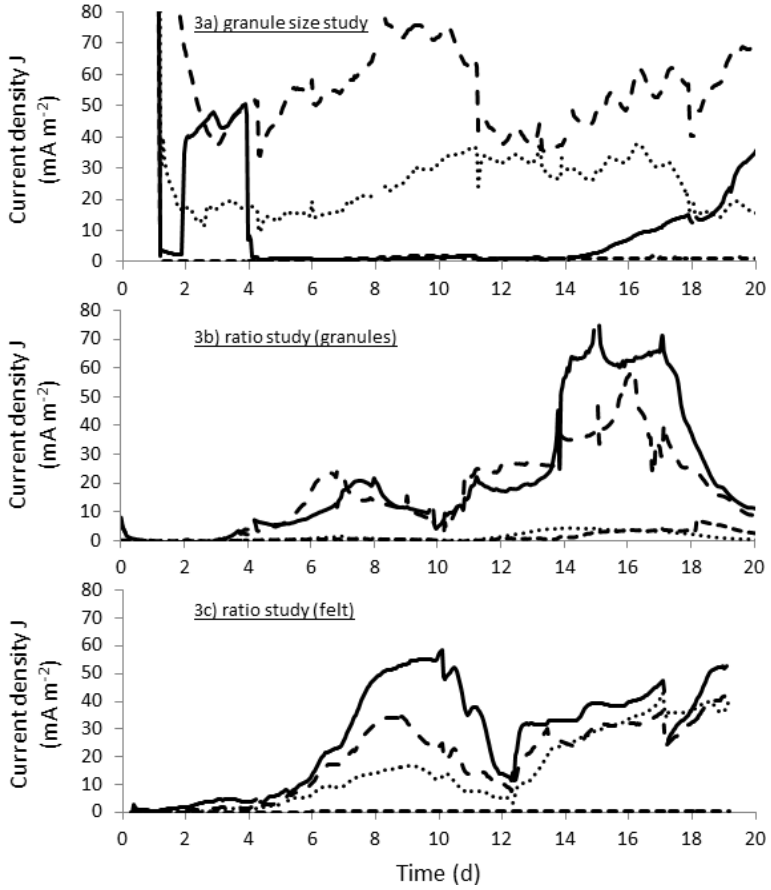



***Figure 2.3*** *Current density profiles over time. a) Granule size study: 1-5 mm (M1a) (····· ), 0.25-0.50 mm (M1b) (– – ·), 0.25-0.50 mm (M2) (——), rod only (- - - -). b) Application ratio study 1-5 mm granules (M1a): AR-0 (- - - -), AR-33 (····· ), AR-67 (– – ·), AR-100 (——). c) Application ratio study 3.18 mm felt (M4): AR-0 (- - - - ), AR-14 (·····), AR-28 (– – · ), AR-42 (——). Data for all tests are averaged for duplicate experiments performed at the same time except for AR-42 felt M4 (3C) which is data of a single experiment. Sudden changes in the graphs are due to manipulations such as polarization curves, sampling or addition of distilled water to the cathode.*

## 3.3 Influence of application ratio

The above described tests were conducted at AR-50, a follow-up experiment was performed to understand the influence of various application ratios on the current density. These tests were conducted for the large granules 1–5 mm (M1a; i.e. the best performing granular material) and for the 3.18 mm felt (M4; a randomly selected felt/cloth material). For the large granules (M1a), the

same current density profile over time could be distinguished for AR-67 and AR-100. The maximum current density obtained was however 59.3 mA $m^{-2}$ for AR-67 while AR-100 obtained 74.8 mA $m^{-2}$ (Figure 2.3b). This coincides with the previous test where AR-50 only reached a maximum current density of 37.9 mA $m^{-2}$ (Figure 2.3a). The felt material test showed an initial clear difference between the different sand/felt application ratios. The higher the amount of felt, the higher the resulting current density. However, addition of COD (day 12,5 Figure 2.3c), resulted in a similar current density profile, 39.6–52.9 mA $m^{-2}$ (Figure 2.3c), for ratios AR-14, AR-28 & AR-42. The highest application ratio still had the highest current density but the other ratios increased considerably in current density after addition of COD. As COD was randomly applied by means of a concentrated solution distributed with a syringe, the effect of locally supplying COD can clearly be seen. This indicates that the system was rather substrate limited starting from day 10. This coincides with the practical implication that electrode needs to be near the source of COD for a sustained current generation in sediment systems (Scott et al., 2008b).

## 3.4 Long term incubation

The short term experiment as described in section 3.2 was prolonged over a longer time period. The microcosms with the small granules (M2) were able to produce a sustainable current, comparable to the ground granules (M1b). This was however mostly due to an increase in cathode potential for M2 (Figure 2.4a). The anode potential remained positive as seen during the short term incubation (Figure 2.4b). It even rose with 0.164 V to + 0.378 V vs. SHE at day 55. The cathode potential increased over the same time period with 0.187 V to 0.521 V vs. SHE. The most striking observation that could be made from the long term data is that the small granules (M2) were not able to obtain a low anode potential, which is necessary for long term sustainable current production, whereas the other materials were able to maintain anode potentials of ~0.2 V vs. SHE when COD was present. The correlation of a low anode potential with COD was observed during the long term incubation where addition of COD resulted in a lowering of the anode potential in open circuit (Figure 2.4b). Adding extra COD did not result in a lower anode potential for material M2. Interestingly, the maximum current that was measured at the end of the forward sweep of the polarization curves of M1b and M2 showed similar patterns over time (Figure 2.4c). Although the maximum current densities were similar between M1b and M2 after 20 days of incubation, the maximum power output was considerably different (Figure 2.4d). M1b had a maximum power output of 37.9 ± 12.9 mW $m^{-2}$ whereas M2 had a maximum power output of 8.0 ± 5.2 mW $m^{-2}$. From these numbers the influence of the higher anode potential can be distinguished as this leads to a lower cell potential and thus a lower power density although similar maximum current densities were achieved.

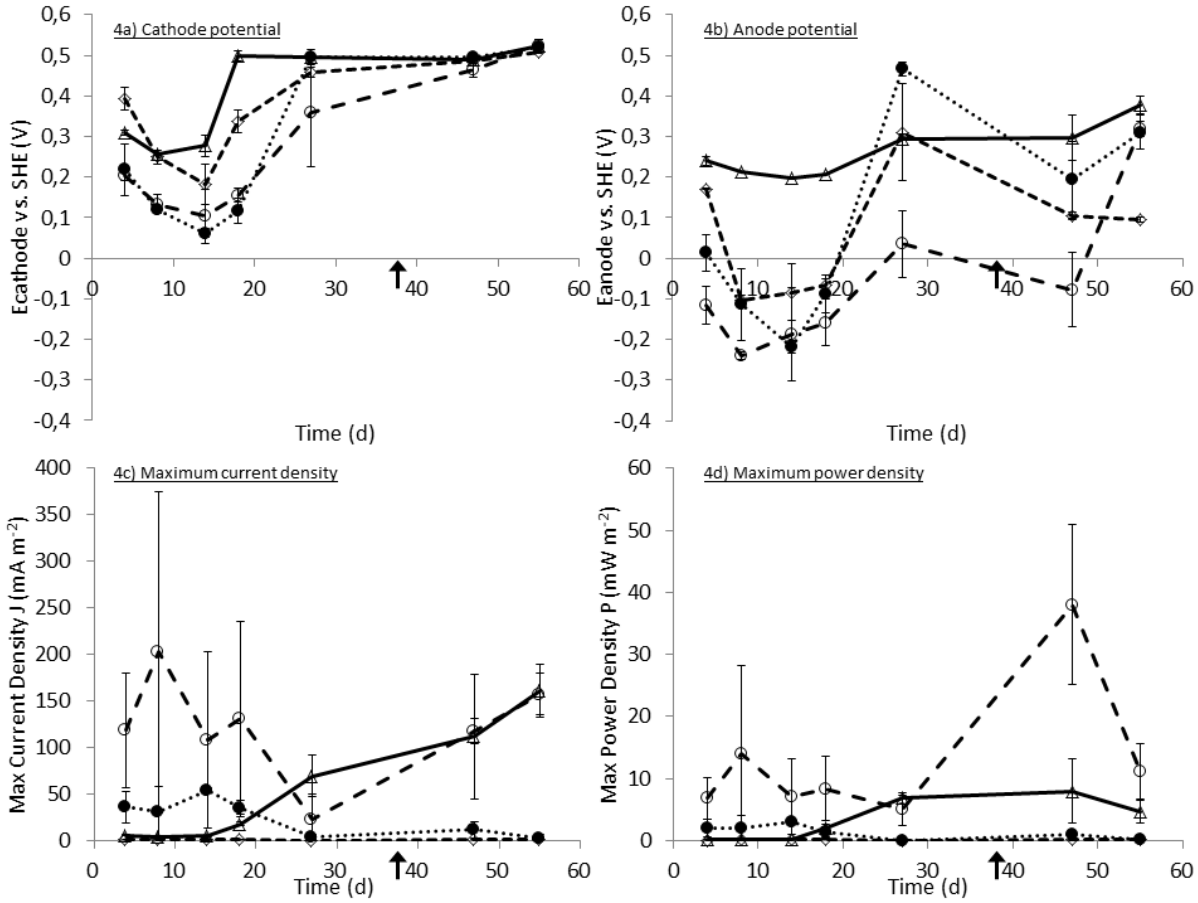



***Figure 2.4*** *Open circuit cathode (4a) and anode (4b) potential over time during granule size study as measured before a polarization curve. Maximum current density i.e. current at short circuit (4c) and maximum power as measured during the forward sweep of the polarization curve (4d): 1-5 mm (M1a) (●), 0.25-0.50 mm (M1b) (○), 0.25-0.50 mm (M2) (△), no material, only the current collector (◇). Error bars indicate standard deviation, sometimes smaller than the symbol (n=2). Arrow indicates addition of COD.*

## 3.5 Organic carbon removal

COD removal efficiencies were determined based on total added COD and produced current. At the end of the short term experiment that investigated the effect of granule size, the highest removal efficiency, 46.5%, was found for the ground granules (M1b), followed by the big granules (M1a) 27.1%, and the small granules (M2) 9.6%. The microcosm with only a rod as current collector obtained a COD removal efficiency of 5.2 %. At the end of the application ratio test for the large granules (M1a) COD removal efficiencies were about equal for AR-67 and AR-100 i.e. 9.8% and 12.1% respectively. AR-0 and AR-33 obtained COD removal efficiencies of 0.71% and 0.77% respectively. For the felt material (M4) COD removed as current was 14.9%, 10.0% and 8.1% of the total added COD for AR-42, AR-28 and AR-14. These overall low removal efficiencies indicate that most organic carbon was removed through an alternative pathway i.e. other than current production. This can be through methanogenesis or by oxidation using an alternative electron acceptor such as residual $O_2$, $NO_3^-$ or $SO_4^{2-}$. As the potential of the anodes indicated anaerobic conditions and there were no N-electron acceptors present (i.e. $NO_2^-$, $NO_3^-$) and $SO_4^{2-}$ could have accounted maximally for 16% of added COD (under the assumption of complete reduction to $H_2S$), it is hypothesized that methane production

was the dominant competing mechanism. The latter gas could however not be monitored in this setup.

## 3.6 Microbial community & material structure

At the end of the long term incubation, samples were analysed for their constituent microbial community based on 16S rRNA gene profiles using DGGE analysis. The results indicated that the type of material had a major influence on the type of microbial community that finally developed. All microbial communities were considerably different from the original electrogenic inoculum, due to the different circumstances experienced in the sediment as compared to the reactor type anode. The communities most similar to the original inoculum were the ones found in the incubations with M1b, with Pearson correlations of 36.2 and 52.5 %. The indigenous sand microbial community was not related to the electrogenic inoculum (0% Pearson similarity). The small ground granules (M1b) had developed a distinct microbial community which could be readily differentiated from the communities on the precursor material (M1a) and on the small granules from another source (M2) (Figure 2.5). Applying ecological parameters to these microbial community profiles shows that the range weighted richness (Rr) is highest for M1b, followed by M1a while M2 shows the lower Rr values.

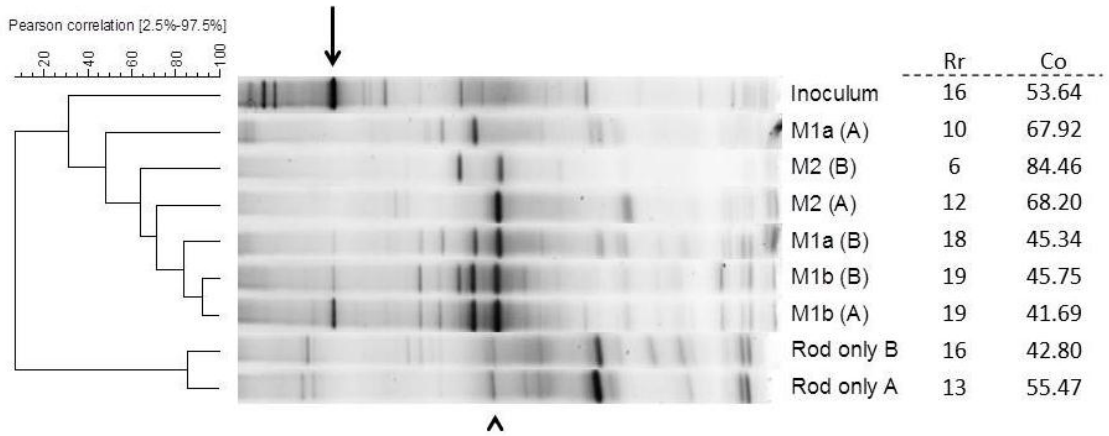



***Figure 2.5*** *Pearson correlation clustering of 16S rRNA gene bacterial PCR products separated on DGGE. Capital 'A' or 'B' indicate duplicate microcosms. Rod only: no electrode material, only a current collector was present i.e. community profile is representative for the sand used. Rr: bacterial richness; Co: community organization. Arrow: dominant microorganism in the inoculum. Arrowhead: non-dominant microorganism in the sand capable of colonizing anode electrodes.*

The community organization (Co) of the microcosms indicated that M2 has the highest value indicating that these microcosms were dominated by a few species whereas the other microcosms were able to sustain a more evenly distributed microbial community. The dominant organism present in the electrogenic inoculum was also found in the best performing microcosms i.e. containing M1b (arrow in Figure 2.5). An organism that was present in the sand was able to colonize the carbon materials abundantly (arrowhead in Figure 2.5). Interestingly, a dense biofilm was not present on any of the granular materials (Figure 2.6). A striking difference could be observed in microbial localization on the electrode material. The microorganisms seemed to prefer to attach to surface imperfections. As the porous bead material M2 had only few of these surface imperfections

compared to M1a and M1b, less interaction between the COD (electron donor), microorganisms and the electrode material (electron acceptor) was possible.

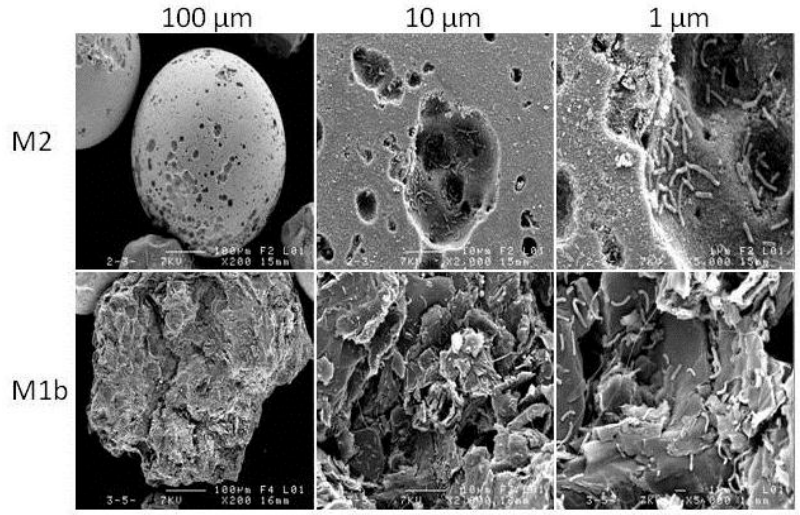



***Figure 2.6*** *Scanning electron microscopic examination of material structure and interaction with microorganisms. The M-numbers refer to Table 2.1. Sizes refer to scale bars in the picture.*

## 4. Discussion

Two parameters influenced the performance of granular carbon materials as anodic electrodes in SMFC. The first important property was the granule size: smaller granules of the same material resulted in a higher performance i.e. maximum 77.7 mA $m^{-2}$ for M1b vs. 37.9 mA $m^{-2}$ for M1a under sustainable current operation. Aelterman et al. (2008b) examined different sized granular material as anode electrode in a packed bed configuration in a reactor type MFC. They did not find any difference in current producing performance between the different granule sizes. They worked with a completely packed bed, while in this study the sand particles were able to interfere with the electrical connectivity between the individual granules. Sand grains can fill up the pore volumes of material M1a due to the 2-40 times smaller size of the grains compared to the carbon material, possibly leading to better electrical contacts between the carbon granules. A more equal mixing is expected between M1b and the grains because of a similar size of both materials. The results show however that the increased surface area per gram of material seems to have a better outcome. These aspects of specific surface, electrical connectivity and tortuosity (i.e. path length of electron movement) and internal resistance need to be further explored with respect to the overall electrical conductivity in the soil.

The second important property are the intrinsic characteristics of the material itself since the same sized materials, but from different precursors, resulted in differing current densities. More markedly, the difference could be seen in the overall anodic potential that was maintained during the incubation. As M2 was made from scratch by carbonizing precursor beads, this material has a highly defined surface structure and internal porosity (Table 2.1). Material M1a and M1b were made of scrap carbon with less defined structural properties and M1b had, due to additional grinding, an even more amorphous nature of the outer surface. The surface structure as examined by SEM had a major influence on where the microorganisms were able to respire with this solid electron acceptor (Figure

2.6). The microbial community present on the electrode material also showed distinct differences in their respective community structures. This can be related to the different electrode materials and can thus explain why the rough material (M1a and M1b) had a better performance in terms of anode potential and thus overall power output. Various theoretical ecological parameters and also visual inspection of the DGGE profile show that M2 creates a more selective environment or a niche environment that excludes important members of the initial electrogenic microbial community. The high anode potential of M2 can possibly be related to trapped oxygen within the highly microporous beads. This means that the microorganisms were able to respire with oxygen, not at the cathode but inside the anode. This idea leads to the new hypothesis that oxygen containing beads might enhance *in situ* bioremediation (see below) through bio-electrochemical removal of pollutants on the outside of the beads. This hypothesis of oxygen trapped inside the beads being the cause for the high anode potential could however not be unequivocally elucidated during this study and warrants further in depth analysis.

The use of granular electrode materials for PMFC has been described previously only in packed bed configurations. De Schamphelaire et al. (2008) noted that the performance of this configuration was inferior as compared to felt mixed with soil. On the other hand, Timmers and co-workers (2010) have noted a good performance with materials from the same supplier as used in this work (M1). Both authors have used different types of plants in their research which might be the cause for the differing performances. The presence of soil can thus enhance current output in a PMFC which justifies mixing the granular electrode with the soil while at the same time creating a high surface area for interaction with plant roots and electroactive microorganisms. Current densities obtained during previous research fit with the current densities as seen during this study. Timmers et al. (2010) obtained 84 ± 70 mA $m^{-2}$ 141 ± 52 mA $m^{-2}$ with their *Spartina anglica* MFC with a packed bed of granules in the anode. De Schamphelaire et al. (2008) were able to obtain a sustainable current density of 120 mA $m^{-2}$ with *Oryza sativa*. For non-planted SMFC current densities are usually smaller than for PMFC (Scott et al., 2008b; Zhang et al., 2010). This leads to the observation that current densities obtained in this study with granular carbon anodes are comparable to previous work and possibly limited by the supply of reactants to and from the anode instead of the anode configuration (Scott et al., 2008b).

From a current density perspective the felt materials seem to be the better choice because with smaller amounts (lower AR value), higher current densities can be obtained compared to granular materials. However, taking also the use of plants and possible agricultural practices into account, this study has shown that granular materials can provide a good alternative. MFC-technology has been proposed as a means of alternative or enhanced soil or sediment remediation (Williams et al., 2010; Yuan et al., 2010; Zhang et al., 2010). The idea behind this concept is to provide an extra electron donor or acceptor in the form of an electrode. This electrode can also be inoculated with useful microorganisms. Till now the MFC technology was only able to work locally as in the soil/sediment , there is little convection and mass transport is diffusion dominated (Scott et al., 2008b). Using a loose, granular anode electrode as opposed to a solid rod or block can provide more degrees of freedom to manipulate the soil or sediment. The anode will not be damaged, merely rearranged and thus increasing the effective working range for a bioremediation process. However the performance of carbon electrode materials under agricultural practices and long term operation in the soil still needs to be evaluated.

## 5. Conclusions

In this work it was shown that granular carbon is able to function as an anode electrode in a sediment microbial fuel cell. For the particular materials in this study it was found that an application ratio of 67% of granules in the soil was most beneficial for the granular material in terms of current output. The application ratio and type of material have a major influence on the resulting current density that can be obtained from the particular SMFC under study. Further work is warranted to better optimise and understand the influence of different materials in combination with different soils on the resulting current density. The effect of the electrode material on the biology present, microorganisms or otherwise needs to be taken into account before this concept can be taken into the field and be applied as a power generation system or for enhancing *in-situ* bioremediation.

## Acknowledgements

This research received funding from the European Community Seventh Framework Programme FP7/2007-2013 under grant agreement no.226532. The authors gratefully acknowledge Matthieu Picot, University de Rennes1, for help with SEM analysis. Tim Lacoere is thanked for help with the molecular biological analysis. The authors thank Jo de Vrieze and Carlos Zamalloa for helpful suggestions and fruitful discussions.

# CHAPTER 3: GREENHOUSE GAS EMISSIONS FROM RICE MICROCOSMS AMENDED WITH A PLANT MICROBIAL FUEL CELL

This chapter has been redrafted after:

Arends JBA, Speeckaert J, Blondeel E, De Vrieze J, Boeckx P, Verstraete W, Rabaey K, Boon N Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell. *In Press: Applied Microbiology and Biotechnology*. DOI: 10.1007/s00253-013-5328-5

## Abstract

Methane ($CH_4$) release from wetlands is an important source of greenhouse gas emissions. Gas exchange occurs mainly through the aerenchyma of plants and production of greenhouse gases is heavily dependent on rhizosphere biogeochemical conditions (i.e. substrate availability and redox potential). It is hypothesized that by introducing a biocatalyzed anode electrode in the rhizosphere of wetland plants, a competition for carbon and electrons can be invoked between electrical current generating bacteria and methanogenic archaea. The anode electrode is part of a bioelectrochemical system (BES) capable of harvesting electrical current from microbial metabolism. In this work, the anode of a BES was introduced in the rhizosphere of rice plants (*Oryza sativa*) and the impact on methane emissions was monitored.

Microbial current generation was able to outcompete methanogenic processes when the bulk matrix contained low concentrations of organic carbon, provided that the electrical circuit with the effective electro-active microorganisms was in place. When interrupting the electrical circuit or supplying an excess of organic carbon, methanogenic metabolism was able to outcompete current generating metabolism. The qPCR results showed hydrogenotrophic methanogens were the most abundant methanogenic group present, while mixotrophic or acetoclastic methanogens were hardly detected in the bulk rhizosphere or on the electrodes. Competition for electron donor and acceptor were likely the main drivers to lower methane emissions. Overall, electrical current generation with BESs is an interesting option to control $CH_4$ emissions from wetlands but needs to be applied in combination with other mitigation strategies to be successful and feasible in practice.

## 1. Introduction

Methane ($CH_4$) emissions to the atmosphere arise from various (a)biotic sources. $CH_4$ has a global warming potential (GWP) of 25 times the GWP of $CO_2$. Therefore these emissions need to be carefully managed (Forster et al., 2007). The main anthropogenic sources of methane emissions are ruminant farming (15-32%) and rice agriculture (9-19%). Taking also natural emissions into account, wetlands and rice agriculture combined amount to 32-47% of total methane emissions (Denman et al., 2007).

Anaerobic decomposition of organic matter resulting in the formation of methane is the consequence of a series of biochemical transformations (Figure 3.1). Bacteria ensure the formation of monomers from complex organic polymers. The monomers are subsequently transformed into various organic acids. In the third step acetic acid, $CO_2$ and $H_2$ are formed which are finally transformed into methane by the hydrogenotrophic or the acetoclastic methanogens (Conrad, 2002; Appels et al., 2008). $CH_4$ formation occurs under specific biogeochemical conditions such as redox potential ($E_h$), pH and ammonia concentration (Conrad, 2002; Majumdar, 2003; Appels et al., 2008; Johnson-Beebout et al., 2009). These parameters give, next to competition for substrate, various handles for control of $CH_4$ production. The main pathway of gas exchange with the rhizosphere under waterlogged conditions is by transport through the aerenchyma of plants (90%). Other mechanisms include diffusion and ebullition (Aulakh et al., 2000; Bazhin, 2010). Therefore a promising route for mitigation of $CH_4$ emissions is to prevent the formation of $CH_4$ in the rhizosphere. Next to introduction of alternative electron acceptors, other mitigation strategies include fertilizer management, water management, biochar addition to the soil and choosing crop varieties with little aerenchyma or little rhizodeposition (Singh et al., 1999; Aulakh et al., 2000; Aulakh et al., 2001; Conrad, 2002; Majumdar, 2003; Zhang et al., 2012).

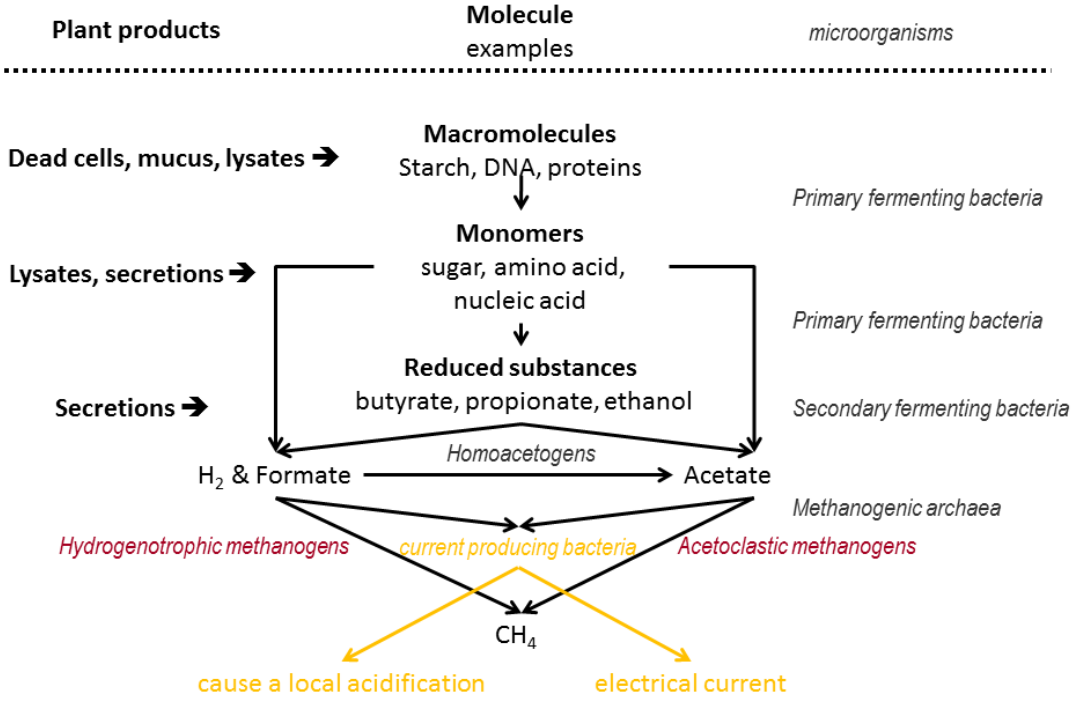



***Figure 3.1:*** *Overview of breakdown of (plant) organic carbon to current and/or methane. Direct competition for substrate as well as indirect by competition by local acidification is indicated. Based on Angenent et al. (2004).*

The last decades microbial extra cellular electron transfer has gained attention on the promise of generating electrical energy directly from various sources of organic matter (Rabaey & Verstraete, 2005). During anaerobic respiration of organic carbon in a microbial fuel cell (MFC), microorganisms are able to use an electrode (the anode) as an electron acceptor. When such a biocatalyzed anode (bioanode) is connected with a (bio)cathode, electrical current can be directly harvested from the microbial decomposition of organic matter in a bioelectrochemical system (BES). The typical substrate for the anodic process is acetate although it has been shown that $H_2$ can also be used (Logan et al., 2006). Waterlogged soils and sediments containing organic matter have been exploited for direct electrical current generation in sediment-MFCs (Reimers et al., 2001; Tender et al., 2008; Donovan et al., 2011). A drawback of sediment-MFCs is the low flux of organic matter towards the anode, limiting high current production. To overcome this issue, plants have been introduced into the anode of a sediment-MFC creating a Plant-MFC which enables sustained current generation from organic matter due to rhizodeposition processes (Strik et al., 2008). An example is the use of rice plants, as they are able to withstand prolonged inundation of their rhizosphere (De Schamphelaire et al., 2008; Kaku et al., 2008).

Methane emissions from rice paddies can be estimated to range from 0 up to 60 mg$CH_4$ $m^{-2}$ $h^{-1}$ (Singh et al., 1999; Gogoi et al., 2005; Xu et al., 2007). This corresponds to the equivalent of an electrical current of 0 to 804 mA $m^{-2}$ (See supplementary information S1 for calculations). Plant-MFCs have been reported to produce a current up to 120 mA $m^{-2}$ (De Schamphelaire et al., 2008). These values are well within the same order of magnitude, therefore it has been hypothesized that current generating metabolism can mitigate $CH_4$ emissions from rice paddy soils and in more general terms, waterlogged wetlands (De Schamphelaire et al., 2008; Ishii et al., 2008; Kaku et al., 2008; Hong et al., 2009; Cabezas De Rosa, 2010). Introducing an electrode (anode) in the rhizosphere of waterlogged plants possibly enables the removal of electrons from the commonly present methanogenic metabolism (De Schamphelaire et al., 2008; Kaku et al., 2008; Cabezas De Rosa, 2010; Arends & Verstraete, 2012). This is analogous to $CH_4$ emission mitigation strategies such as the addition of ferric iron or sulphate to stimulate iron reducing or sulphate reducing microorganisms in their competition for reducing equivalents with methanogens (Liesack et al., 2000; Conrad, 2002).

Anodic oxidation of organic matter leads to a decrease in pH during current generation (Rozendal et al., 2006a; eq. S3.2). Methanogenic metabolism can be inhibited by a decrease in pH (Appels et al., 2008). Acidification due to current generating metabolism can possibly form a second route to inhibition of methanogenic activity, next to competition for substrate.

Cabezas de Rosa (2010) has observed a 47% decrease in pore water $CH_4$ concentrations in a rice paddy soil sediment-MFC without plants. Kaku et al. (2008) were not able to show lower $CH_4$ emissions in closed circuit operation of a Plant-MFC during a field trial in a rice paddy. In this work the results of a microcosm study are presented in which rice plants were grown in vermiculite with a BES in the rhizosphere. The aim of this work was to investigate 1) the influence of the external resistance 2) the mechanism and 3) the archaeal pathways involved in possible methane emission mitigation in Plant-Sediment MFC rhizosphere/anode environments.

## 2. Materials and Methods

### 2.1 Microcosm setup

All experiments were carried out in microcosms consisting of Perspex tubes (14 cm inner diameter, 35 cm high). The bottom 15 cm contained 12 sample ports at various heights while the top 10 cm contained 4 sample ports (Figure 3.1, S3.2). The containers were filled with graphite granules (type 00514, Mersen, Belgium) and vermiculite (Nestaan, Belgium) in a volume ratio of 2:1, to a height of 18 cm. This ratio was chosen in order to have a good electrical conductivity in the rhizosphere (Arends et al., 2012) and to ensure that only added organic carbon (COD) or rhizodeposition were the sources of organic carbon. On top of this mixture, a layer of 3 cm vermiculite was added which prevented electrical contact between the anode and the cathode. Vermiculite and not rice paddy soil was chosen to create uniform rhizosphere conditions and to be able to measure and/or control the amount of organic carbon in the rhizosphere/anode as much as possible. The sides of containers were covered with black tape to create a dark root environment. At 5 and 15 cm height, a piece of graphite felt (10*10*0.3 cm; Alfa Aesar, Germany) interwoven with a carbon rod (5 mm diameter; Morgan, Belgium) was placed as the anodic current collector. The cathode was made of two sections of 5*10*0.3 cm graphite felt interwoven with a carbon rod. The two sections were placed so that the plants were able to grow in the middle of the Perspex tube. A watertight connection between the wire and the carbon rod was established by means of conductive carbon cement (CCC, Leit-C; Fluka, Germany) and vulcanizing tape. An Ag/AgCl reference electrode (BASi, United Kingdom) was placed at 10 cm height in the rhizosphere/anode compartment and also in the cathode compartment. To be able to measure soluble pore liquid components, a rhizon sampler was located at 6.7 and 16.7 cm height (10 cm porous, Rhizosphere Research Products, The Netherlands). In order to collect gas emissions, the top of the Perspex container contained a ledge for a water lock. This ledge was able to support a tube of 12.3 cm inner diameter and 1 m height (Figure S3.2). The top of this tube contained four connectors for sampling or other uses. One connection was occupied by the electrical connections for a fan to mix the contents of the headspace; another was used for temperature measurements during headspace gas sampling. All sampling i.e. pore liquid, trace gas and polarization curves was performed at the same time for a microcosm during the whole experimental period to minimize circadian (day/night) influences on the results. For the exploratory study, additional square containers were used with the same configuration except for the surface area (18*18 cm). In experimental setups meant for open circuit conditions, only 1 anode and 1 rhizon sampler were placed in the container. An overview of all microcosms and experimental conditions can be found in table S3.1.

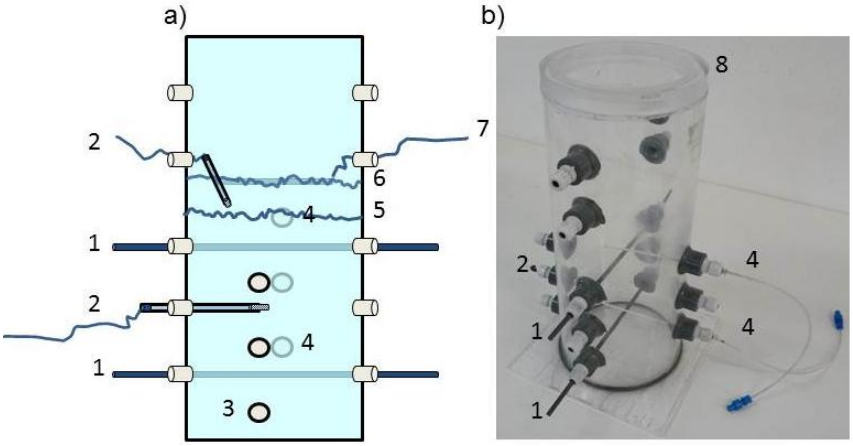



***Figure 3.2:*** *a) Schematic overview of microcosm, not to scale. b) photograph of empty microcosm. 1) anode current collector, 2) Ag/AgCl reference electrode, 3) port for liquid addition, 4) positions for Rhizon samplers, 5) vermiculite level, 6) water level, 7) cathode electrode and wire, 8) rim to hold gas cover.*

## 2.2 Rice plants & rhizosphere organic carbon

Rice seeds (*Oryza sativa*, ssp. *indica*, cultivar C101 PKT) were germinated in ¼ Hoagland nutrient solution at 28°C in the dark (De Schamphelaire et al., 2008). The seedlings were subsequently transplanted into the Perspex containers, which was considered the start of the experiment. Two seedlings were planted per microcosm. The microcosms were placed in a light (12/12 light/dark using Osram 400W plantastar and Osram 400W powerstar daylight HQI-BT Lamps) and temperature (30 ± 2 °C) controlled growth room. Development of the plants was scored according to Counce et al. (Counce et al., 2000). To maintain a constant water level above the vermiculite, plants were regularly watered with tap water and once per week with ½ Hoagland nutrient solution. Upon good visible plant development, nitrate was removed from the Hoagland nutrient solution (Helder et al., 2012). Dried and ground barley straw was added to the rhizosphere as additional organic carbon in the exploratory study (Table S3.1). Soluble organic carbon mimicking exudation was added to the rhizosphere in the main study as an equal weight mixture of acetate, starch, glucose, malate and succinate. The amount of organic carbon added was calculated to attain approximately 30 mg $CH_4$ $m^{-2}$ $h^{-1}$ emission resulting in an organic carbon addition rate of 2.9 gCOD $m^{-2}$ $d^{-1}$ (~ 401 mA $m^{-2}$; starting from day 39; See supplementary information S3.1.).

## 2.3 Electrochemical analysis

Cell potential over an external resistor (500 Ω, unless stated otherwise), anode and cathode potentials ($E_{an}$ respectively $E_{cath}$) versus a reference electrode were measured continuously at 5 min intervals (HP 34970A, Agilent). The potential of the Ag/AgCl reference electrodes were regularly monitored relative to a calomel electrode (+244 mV vs. Standard Hydrogen Electrode (SHE); QIS, the Netherlands) for correct conversion of the electrode potentials compared to the SHE. Electrode potentials are consequently reported versus the standard hydrogen electrode. Polarization and power curves were recorded on a weekly basis with a Bistat potentiostat (Biologic, France) at a scanrate of 1 mV $s^{-1}$ following a 20 min stabilization period in open circuit. Electrochemical calculations were performed according to Rabaey et al. (2005c) and are based on hourly averages.

Current and power density are reported normalized to the plant growth area (0.015 or 0.032 $m^2$; Table S3.1).

## 2.4 Chemical analysis

Liquid and headspace gas samples were taken twice a week. Anions ($PO_4^{3-}$, $NO_3^-$, $NO_2^-$, $SO_4^{2-}$) in the pore liquid were analysed using a metrosep A Supp 5-150 column after a metrosep A 4/5 guard column in a 761 Compact IC with a conductivity detector (Metrohm, Switzerland). Pore liquid soluble chemical oxygen demand (COD) was determined using commercial kits according to the manufacturer's instructions (Machery-Nagel, Germany). Volatile fatty acids (VFA) were determined, after etheric extraction of 2 mL pore liquid, on an EC-1000 Econo-Cap column in a gas chromatograph (2014, Shimadzu) with a flame ionization detector (FID). Pore liquid and overlying water pH and conductivity (EC) were determined using a handheld probe (SP10B and SK20, Consort, Belgium). Headspace air temperature was monitored during gas sampling.

## 2.5 Trace gas analysis

Twice a week the headspace of the microcosms was closed to determine gas emissions from the rhizosphere-plant continuum. Every 30 min. duplicate 15 mL gas samples were taken from the headspace over a period of 2 hours. A duplicate 15 mL background air sample was taken to account for gas concentrations already present in the rice cultivation room. All gas samples were stored in a 12 mL vacutainer, allowing at least 3 subsamples for analysis. Vacutainers were stored in the dark at ambient temperature (20 ± 2 °C) until analysis. $CH_4$ and $CO_2$ were analysed using a gas chromatograph (Trace GC Ultra, Thermo Fisher Scientific, Germany). Methane was determined using FID while $CO_2$ was determined using a thermal conductivity detector (TCD). Nitrous oxide was measured using a gas chromatograph (14B, Shimadzu, Japan) with a 63Ni electron capture detector (ECD).

## 2.6 PCR-DGGE and qPCR

At the end of the experimental period, samples were taken for microbial community analysis. Sample positions were: anode current collector, anode felt (top & bottom) and anode granules with and without rice roots (Figure S3.2). Anode granules without rice roots are considered as bulk vermiculite. Total DNA was extracted using the method as described by Boon et al. (Boon et al., 2000). The 16S rRNA-gene was amplified using PCR (Table 3.1). The bacterial community structure was visualized by means of denaturing gradient gel electrophoresis (DGGE) of the obtained PCR amplicons using an 8% polyacrylamide gel and a denaturing gradient of 40-60% on an INGENY phorU2X2 system for 16 h at 120 V (Goes, The Netherlands). The gel was stained with Sybr green in 1x TAE buffer. The resulting community structures were analysed using BioNumerics software v5.1 (Applied Maths, Belgium). DGGE profile similarities were based on band based clustering using the Jaccard coefficient to minimize the influence of background subtraction on the clustering. Theoretical ecological parameters, community organization (Co) and richness (Rr), were used to numerically describe the bacterial communities. In brief, Co describes the species abundance distribution based on the gini coefficient of the microbial community. Rr describes the species richness of the microbial community and can be interpreted as the carrying capacity of a certain environment (Read et al., 2011). The archaeal community structure was analysed by means of qPCR (Table 3.1 & S3.2) using

the GoTaq qPCR MasterMix (Promega, Belgium) on a StepOnePlus qPCR machine and accompanying software (Applied Biosystems, United Kingdom).

***Table 3.1:*** *(q)PCR primers and conditions used for elucidating the microbial communities. D: denaturation, A: Annealing, E: Elongation phases of the PCR cycle. For the specific methanogenic groups, taxonomic level and major metabolism are indicated.*



| Reaction | Target | Primers | Program | Reference |
|---|---|---|---|---|
| PCR-DGGE | Bacterial 16S rRNA gene | 338F-GC<br>518R | D: 94°C, 300s<br>30 cycles<br>D: 95°C, 60s<br>A:53°C, 60s<br>E: 72°C, 120s<br>E: 72°C, 600s | (Ovreas et al., 1997; Boon et al., 2000) |
| qPCR | Total Bacteria 16S rRNA gene | 338F<br>518R | D: 94°C, 10min<br>40 cycles<br>D: 94°C, 15s<br>A&E: 60°C, 60s | |
| | Total Archaea 16S rRNA gene | ARC787F<br>ARC1059R | D: 94°C, 10min<br>40 cycles<br>D: 94°C, 10s<br>A&E: 60°C, 60s | (Yu et al., 2005) |
| | *Methanosaetaceae*<br>Family (acetoclastic) | Mst 702F<br>Mst 862R | See total archaea | |
| | *Methanosarcianceae*<br>Family (mixotroph) | Msc 380F<br>Msc 828R | See total archaea | |
| | *Methanobacteriales*<br>Order ($H_2$/$CO_2$) | MBT 857F<br>MBT 1196R | See total archaea | |
| | *Methanomicrobiales*<br>Order ($H_2$/$CO_2$) | MMB 282F<br>MMB 832R | D: 94°C, 10min<br>40 cycles<br>D: 94°C, 10s<br>A&E: 63°C, 60s | |

## 3. Results

A first set of exploratory experiments was used to determine the key operational parameters (electrode position, organic matter content and initial microbial community) relevant for $CH_4$ emissions. A second, more detailed study focused on the effect of placing a sediment MFC in the rhizosphere of rice plants on greenhouse gas emissions.

### 3.1 Exploratory study

From the exploratory study no clear difference could be distinguished in methane emissions between closed and open circuit conditions as well as between planted and sediment conditions. Methane emissions averaged across the open (n=7) and closed circuit (n=3) configurations (table S3.1) were 157 ± 76 vs. 169 ± 68 $mgCH_4\ m^{-2}\ h^{-1}$ respectively. These values are high compared to natural field conditions (0-60 mg $CH_4\ m^{-2}\ h^{-1}$ (Singh et al., 1999; Gogoi et al., 2005; Xu et al., 2007)). The microcosm that was not supplemented with straw was the configuration that came close to natural $CH_4$ fluxes in the order of 35 $mgCH_4\ m^{-2}\ h^{-1}$. Interestingly, the exploratory study revealed that

interrupting the current flow from a well performing Plant-MFC resulted in a doubling of $CH_4$ emission flux (Figure S3.3). This doubling was about 20 times higher than the loss in electrons due to current (See supplementary information for calculations). This suggests the influence of changing electrochemical or thermodynamic conditions, possibly related to local acidification close to the anode electrode, next to direct competition for electrons between the current generating and methanogenic microbial populations. Based on the results of the exploratory work, it was chosen to work in the detailed study with no added organic carbon at the start of the experiment and also to not add methanogenic sludge.

The next sections provide the results of the detailed study.

## 3.2 Plant growth characteristics

4 Microcosms were set up for the detailed study. One microcosm (M4) lagged behind in development rate in the first growth phase, probably due to leakage of copper from the current collector wire at the cathode i.e. at the top of the microcosm. This was amended by replacing the wire and removing the affected vermiculite, after which the rate of development of the plants in M4 as well as electrochemical and gas emissions were similar to comparable microcosms. Final above ground dry biomass were M1: 9.5 ± 1.2 gDW plant $^{-1}$ M2: 10.9 ± 0.1 gDW plant$^{-1}$ M3: 11.5 ± 1.7gDW plant$^{-1}$ M4: 4.3 ± 2.2 gDW plant$^{-1}$.

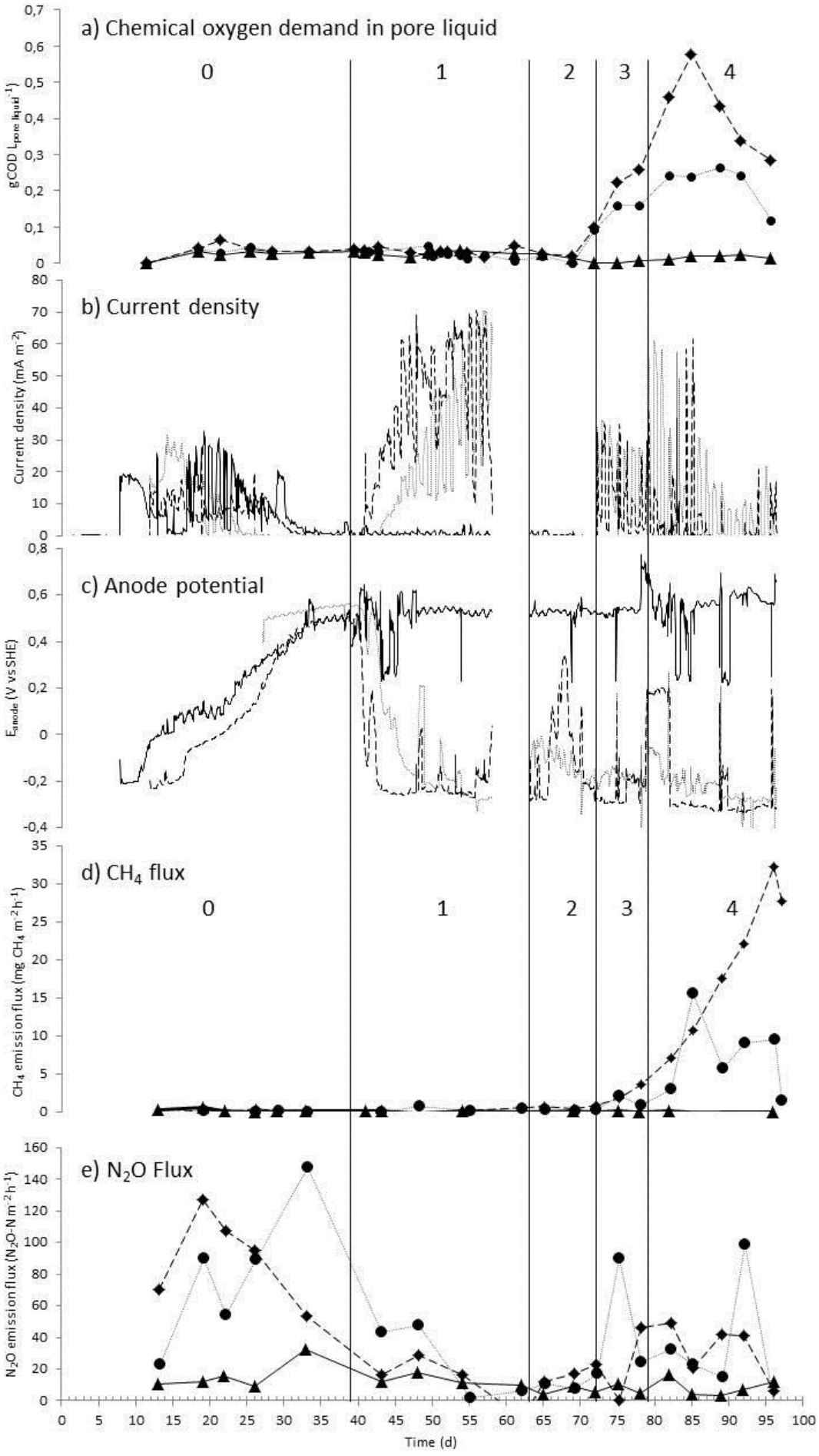
## 3.3 Current, power and electrochemical performance of the Plant-MFCs

Cell potentials of all four plant-MFCs increased during the first 21 days after planting to an average maximum value of 0.23 ± 0.039 V resulting in a current density of 27.9 ± 4.2 mA m$^{-2}$ (Figure 3.2b). After day 21, the cell potentials decreased. Addition of organic carbon (starting at day 39) resulted in an increase in current density for M3 and M4 (days 45-55) to 45.5 ± 11.8 mA m$^{-2}$ for M3 and 25.9 ± 13.7 mA m$^{-2}$ for M4 (Figure 3.2b). M1 and M2 did not receive any organic carbon (Table S3.1). Consequently, no increase in current nor in greenhouse gas emissions were observed. M2 is not plotted in figure 2 as data were similar to M1. The high standard deviations are due to a circadian rhythm with daily maxima up to 69.6 mA m$^{-2}$ and 62.4 mA m$^{-2}$ for M3 and M4 respectively and nightly minima as low as 22.9 mA m$^{-2}$ and 9.7 mA m$^{-2}$ for M3 and M4 (Figure 3.3). After the open circuit period (day 63-72), the current density did not recover up to these values. Attempts to increase current density by lowering the external resistance did not result in an increased current flow (Figure 3.2b). The main cause for the low current density was a deterioration of the cathode performance as evidenced by the cathode potential ($E_{cath}$). While the cathode potential reached daily maxima of 0.84 V and 0.62 V for M3 and M4 and nightly minima of 0.07 V and 0.17 V during days 45-55 (Figure 3.3), daily cathode maxima went only up to 0.40 V and 0.37 V for M3 and M4 upon re-closing the opened electrical circuit with a 500 Ω resistor. Decreasing the external resistance to 100 Ω (d 79) resulted in an average (including day & night) $E_{cath}$ over the remainder of the experimental period of -0.11 ± 0.13 V for M3 and -0.11 ± 0.07 V for M4. The anode potential of M3 and M4 remained low after day 39, -0.20 ± 0.16 V for M3 and -0.19 ± 0.09 V for M4 (Figure 3.2c and 3.3). Variations in anode potential are mainly caused by polarization curves (invasive technique) or sampling. Overall average power production during days 45-55 resulted in 22.2 ± 9.5 mW m$^{-2}$ for M3 and 6.6 ± 6.6 mW m$^{-2}$ for M4. Here again high standard deviations can be seen, due to the circadian rhythm influencing cathode performance. From the polarization and power curves the maximum attainable current and power can be estimated. The maximum power output was attained at day 61, 72 mW m$^{-2}$ for M3 and 56

mW $m^{-2}$ for M4. The maximum short circuit current generated during the polarization measurements was 0.58 A $m^{-2}$ for M3 at day 53 and 0.20 A $m^{-2}$ for M4 at day 61. For M1 and M2, the maximum values amounted to 9 mW $m^{-2}$ and 1 mW $m^{-2}$ respectively, obtained at day 19.



***Figure 3.2:*** *(next page) overview of a) chemical oxygen demand, b) current density, c) anode potentials, d) methane flux, multiply value by 25 to arrive at $CO_2$ equivalents and e) nitrous oxide flux, multiply value by 298 to arrive at $CO_2$ equivalents during the rice growth period. M1: ▲ M3: ◆ - M4: ●. M2 is not plotted as data are similar to M1. For continuous measurements, b) and c), symbols are omitted. Experimental stages: 0: initial plant growth and MFC start-up 1: addition of organic carbon 2: open circuit period 3: closed circuit period, 500 Ω 4: external resistance lowered from 500 Ω to 100 Ω for M3 and M4. No electrochemical data available for day 58-63.*


a) Chemical oxygen demand in pore liquid
0
1
2
3
4
gCOD L$_{pore\ liquid}^{-1}$)
b) Current density
Current density (mA m$^{-2}$)
c) Anode potential
E$_{anode}$ (V vs SHE)
d) CH$_4$ flux
CH$_4$ emission flux (mg CH$_4$ m$^{-2}$h$^{-1}$)
e) N$_2$O Flux
N$_2$O emission flux (N$_2$O-N m$^{-2}$h$^{-1}$)
Time (d)

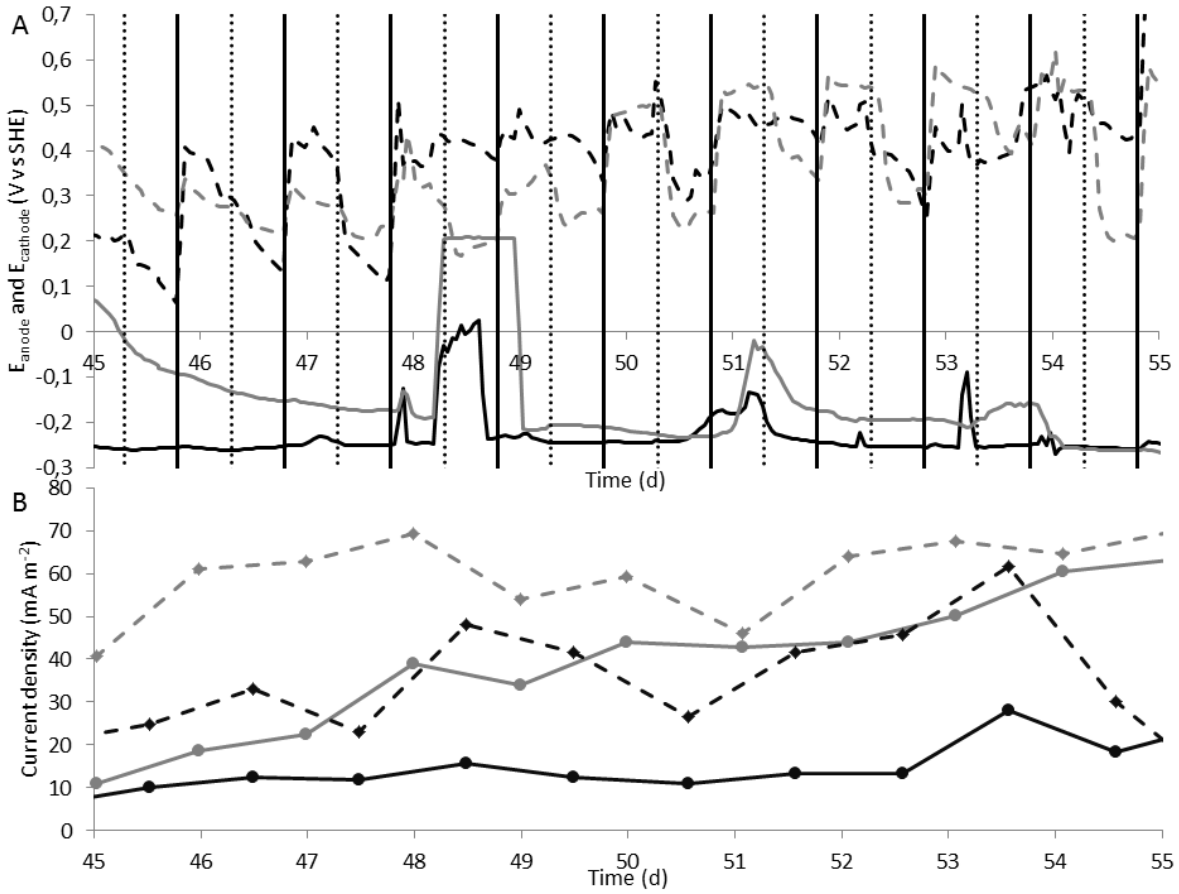



***Figure 3.3:*** *Close up of data between day 45-55 showing the diurnal variation. a) anode and cathode potentials during day 45-55. Day 48: anode reference electrodes disconnected. Vertical solid lines; 7 am, lights on. Vertical dotted lines; 7 pm, lights off. M3 anode: M3 cathode: M4 anode: M4 cathode: b) Current density resulting from the varying electrode potentials day 45-55. M3 daily max.: M3 Nightly min. : M4 daily max.: M4 Nightly min. :* .

## 3.4 Current was generated after addition of organic carbon

The overall availability of organic carbon in the pore liquid was low during the first part of the experiment (37.0 ± 18.1 mgCOD $L^{-1}$ for M3 and 31.2 ± 18.7 mgCOD $L^{-1}$ for M4 till day 72; Figure 3.2a). To compensate for the low reducing power provided by the plants, soluble organic carbon was added to mimic the rhizodeposition process. The added COD was immediately used by the electroactive microbial community present on the anode, as can be seen by the almost instantaneous increase in current (Figure 3.2b). The organic carbon was efficiently oxidized as only from day 72 it started to appear in the pore liquid of the bulk (Figure 3.2a). No short chain fatty acids were detected in the bulk liquid of the microcosms (data not shown). The efficient use of exogenous organic matter was not only for current generation, as current generation only accounted for maximum 15% of the organic carbon ([~60 mA $m^{-2}$] / [401 mA $m^{-2}$] *100%). The organic carbon that was not used for current generation accumulated in the system or was metabolised using alternative electron acceptors such as nitrate and sulphate or $O_2$ due to radial oxygen loss from the rice roots. For M3 and M4 a decrease of 69 % and 75 % COD equivalents was noticed for nitrite, nitrate and sulphate combined after day 39. The substrate limited situation of all microcosms could also be derived from the fact that M2 received the same nutrient solution as M3 & M4 but without organic carbon. The

addition of only Hoagland solution did not result in an increase of current, nor $CH_4$ emissions nor organic carbon concentration in the pore liquid. M1 did not receive any extra organic carbon or liquid, which also did not result in an increase of available organic carbon in the pore liquid.

Due to current generating metabolism, a drop in pH could be expected but this was not detected in the bulk pore liquid. The pH of all pore liquid samples was 7.79 ± 0.44 over the whole experimental period.



## 3.5 Anode biofilm redox potentials in relation to greenhouse gas emissions

Methane was produced when favourable redox conditions existed for the producing microbial community (i.e. < -150 mV vs. SHE; (Hou et al., 2000; Yu & Patrick, 2003; Johnson-Beebout et al., 2009). The potentials measured in the reference electrode/ bioanode combination could be related with bulk gas emissions for $CH_4$ (Figure 3.4a). $N_2O$ emissions showed a less clear relationship with the redox potentials as measured with the reference electrode/ bioanode combination (Figure 3.4b).

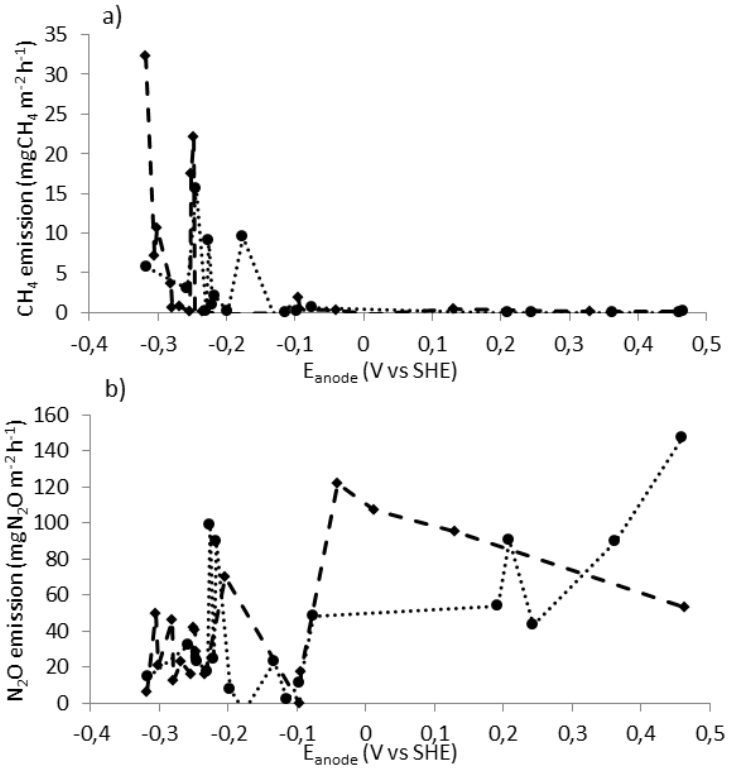



***Figure 3.4:*** *Greenhouse gas emissions in relation to measured anode potential. a) $CH_4$ multiply value by 25 to arrive at $CO_2$ equivalents b) nitrous oxide flux, multiply value by 298 to arrive at $CO_2$ equivalents. M3: ◆ - - M4: ●.*

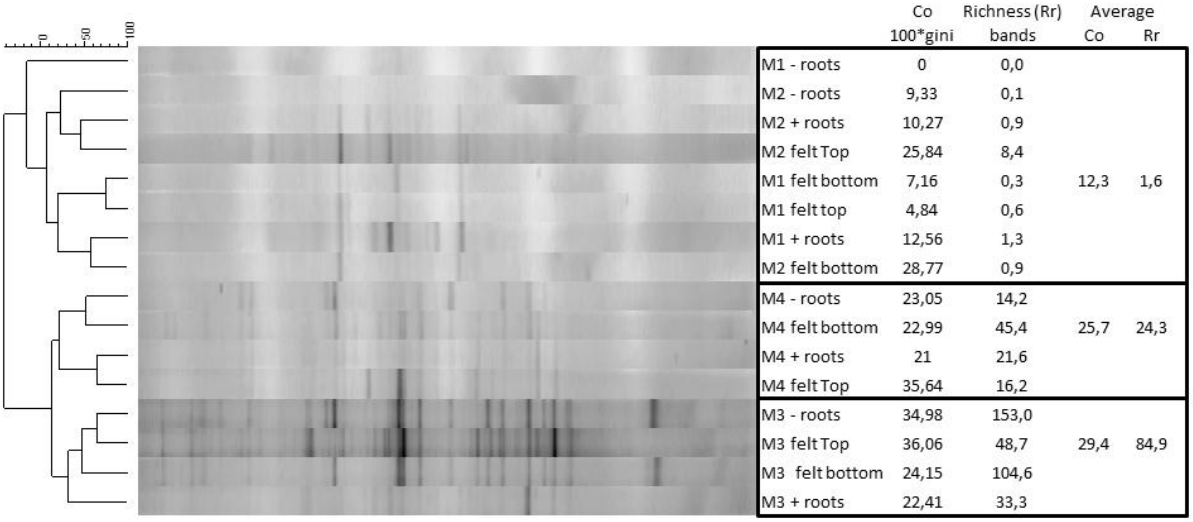
## 3.6 Methane emissions lagged behind current generation

Organic carbon was efficiently metabolised to current, to other sinks or accumulated, but was not metabolised to methane. Methane only appeared in the headspace measurements of M3 & M4 after the current flow was interrupted by removing the external resistor from the electrical circuit for 9 days (day 63-72). Towards the end of this period (day 69; Figure 3.2a), organic carbon concentrations started to increase in the pore liquid (up to 577 mgCOD $L^{-1}$ for M3, day 85 and 265 mgCOD $L^{-1}$ for

M4, day 89). This increase was followed by an increase in $CH_4$ emissions (starting on day 75; Figure 3.2d). Closing the electrical circuit with a 500 Ω resistor on day 72 did not result in a decrease in $CH_4$ emissions. Conversely, $CH_4$ emissions increased even further. Methane release from the microcosms did not show any relation with either closing the electrical circuit or lowering the external resistance to 100Ω (day 79; Figure 3.2b and 3.2d). The effect of the electrical current on the $CH_4$ emissions after re-closing the external circuit remains to be determined as the current was markedly lower compared to the period before the electrical circuit was opened. This was the result of the low activity of the (bio)cathode resulting in an overall low current density (Figure 3.2b and § 3.3). Methane emissions in these microcosms were indeed facilitated by the aerenchyma of the rice plants. This was established by harvesting the above-ground biomass at the end of the study period and subsequently determining the $CH_4$ flux. Cutting the biomass in M3 and exposing the aerenchyma to the air resulted in a 14% lower emission the next day (32.3 to 27.8 $mgCH_4\ m^{-2}\ h^{-1}$). Cutting the biomass in M4 and raising the water level to above the aerenchyma resulted in a 85% decrease of emissions the next day (9.5 to 1.4 $mgCH_4\ m^{-2}\ h^{-1}$).

## 3.7 Bacterial community structure is driven by available organic carbon

Based on the DGGE-profile of the 16S rRNA gene of the bacterial community, the main parameter affecting the enrichment of the various microbial communities was the amount of available electron donor (organic carbon) present at the various locations. This was confirmed by two methods of cluster analysis, Jaccard band based clustering (Figure 3.5) and via a Pearson correlation clustering (not shown). M3 and M4 were able to support a bacterial community with a higher community organisation (Co) and a higher richness (R) compared to M1 and M2. The higher richness indicates a more developed microbial community. It can be reasoned that this is related to a metabolic network where fermentation of substrates to $H_2$ plays a key role however this could not be validated with the used technique.




| | Co 100*gini | Richness (Rr) bands | Average Co | Average Rr |
|---|---|---|---|---|
| M1 - roots | 0 | 0,0 | | |
| M2 - roots | 9,33 | 0,1 | | |
| M2 + roots | 10,27 | 0,9 | | |
| M2 felt Top | 25,84 | 8,4 | | |
| M1 felt bottom | 7,16 | 0,3 | 12,3 | 1,6 |
| M1 felt top | 4,84 | 0,6 | | |
| M1 + roots | 12,56 | 1,3 | | |
| M2 felt bottom | 28,77 | 0,9 | | |
| M4 - roots | 23,05 | 14,2 | | |
| M4 felt bottom | 22,99 | 45,4 | 25,7 | 24,3 |
| M4 + roots | 21 | 21,6 | | |
| M4 felt Top | 35,64 | 16,2 | | |
| M3 - roots | 34,98 | 153,0 | | |
| M3 felt Top | 36,06 | 48,7 | 29,4 | 84,9 |
| M3 felt bottom | 24,15 | 104,6 | | |
| M3 + roots | 22,41 | 33,3 | | |

***Figure 3.5:*** *Jaccard bandbased clustering of 16s rRNA-gene based microbial community. Microbial Resource Management (MRM) parameters community organization (Co) and range weighted richness (Rr) are indicated (Read et al. 2011).*

### 3.8 Archaeal community structure

The results of the qPCR analysis of the archaeal microbiome at the end of the experiment indicates that $H_2$ based methanogenesis (orders *Methanobacteriales* & *Methanomicrobiales*) was likely the dominant process compared to acetate based methanogenesis (Family *Methanosaetaceae*). M1 (low COD concentration in the pore liquid, and resulting low $CH_4$ emissions) showed a slight enrichment in $H_2$ consuming *Methanomicrobiales* compared to acetate consuming methanogens (3.9 vs. 3.8 log copies $g^{-1}$, other groups were below detection) at the roots of the rice plants. The microcosms with a higher COD-load (M3 & M4) showed at all sample locations (with and without roots, top and bottom felt) a higher abundance of $H_2$-consuming methanogens. *Methanosaetaceae* (acetate dependent) were present in a lower abundance compared to *Methanosarcinaceae* (mixotrophic) at the bottom anode (5.2 vs. 6.0 log copies $g^{-1}$ for M3 & 4.7 vs. 5.1 log copies $g^{-1}$ M4) whereas they were more abundantly present at the top anode (5.0 vs. 4.7 log copies $g^{-1}$ for M3 & 4.7 log copies $g^{-1}$ vs. < 3.7 log copies $g^{-1}$ for M4). All archaeal groups were below detection limit at all sample locations for M2, correlating with the low COD concentrations in the rhizosphere. The same was valid for M1 where archaea were only detected in samples with roots. The copy numbers of all four groups of methanogens added up to the copy numbers obtained by means of the total archaea qPCR for all microcosms. This indicates that no other groups were involved in methanogenesis in these microcosms.

## 4. Discussion

### 4.1 Anode/rhizosphere organic carbon and redox potential

The low current density recorded for M1 and M2 over the whole experimental period and for M3 and M4 for the first 40 days indicate that exudation of low molecular weight organic acids or sugars were not directly responsible for current generation at the anode. Moreover, no short chain fatty acids were detected in the bulk liquid, indicating that other organic components were the major components of the organic material present. These results are in line with previous research where long start-up times for Plant-MFCs were recorded with pristine anodes (De Schamphelaire et al., 2008; Helder et al., 2010; Helder et al., 2012). The long start-up times indeed suggest that components derived from rhizodeposition other than small molecules released via direct exudation are more important in current generation by plant-sediment MFCs. The model put forward by Timmers et al. (2011) showed that a low amount of reducing equivalents available for current generation was corroborated by the presence of alternative electron acceptors and could possibly be related to radial oxygen loss into the rhizosphere (Liesack et al., 2000; Timmers et al., 2011). In this work, redox potentials as measured at the anode electrode of M3 and M4 decreased over time, with subsequent increase in bulk gas emissions of $CH_4$, with increased current generation and a decrease in alternative electron acceptor concentrations. For both $CH_4$ and $N_2O$, favourable redox potentials are needed, i.e. for $N_2O$ $E_h$ >180 mV vs. SHE via denitrification and for $CH_4$ $E_h$ < -150 mV vs. SHE (Hou et al., 2000; Yu & Patrick, 2003; Johnson-Beebout et al., 2009). Bulk methane emissions can be related to redox potentials as determined with the bioanodes. A clear relation was not observed for $N_2O$ emission and bulk anode potentials (Figure 3.4). This can be attributed to the position of the reference electrode in relation to the location of microbial activity. The top of the microcosm which was exposed to ambient air, while the bulk microcosm was waterlogged.

## 4.2 Current producing metabolism precedes methanogenic metabolism

As stated before, organic carbon was instantaneously metabolised to current and not to $CH_4$. Methane emissions became apparent after interrupting current flow by removing the external load. During this period, organic carbon became available in the pore liquid and $CH_4$ emissions started to increase. An exploratory study already revealed that at higher organic carbon loads, no difference in methane emissions could be observed between the open and closed circuit conditions. In accordance with the findings in this work, Freguia et al. (2008) have also shown that methanogenesis is a robust process largely operating independent of electrode based processes. This was established in a reactor type anode with a packed bed of similar granular electrode material. Kaku et al. (2008) were also not able to detect decreased $CH_4$ emissions from Plant-MFCs in closed circuit in a rice field. On the other hand, it was shown that a closed circuit MFC was able to release 10 times less methane compared to an open circuit MFC using a reactor type setup and rice paddy soil as anode inoculum (Ishii et al., 2008). Cabezas de Rosa (2010) showed that it was possible to reduce methane emissions with electrodes in sediment systems by 47%. These seemingly contradicting observations indicate that the electrical circuit and the current generating microorganisms were only able to outcompete methanogenic microorganisms at low organic carbon concentrations (electron-donor) and high electron acceptor concentrations (anode in closed circuit). This concept is supported by the fact that the affinity (Ks) of *Geobacter sulfurreducens* (a known current generating microorganism) for acetate can be as low as 10 µM whereas the lowest affinity for acetate reported for *Methanosaetaceae* is 160 µM (Esteve-Núñez et al., 2005; Qu et al., 2009). For *Methanosarcinaceae* this value is even higher, 3 mM (Qu et al., 2009).

Competition for substrate seemed to be most important as acidification of the bulk pore liquid was not detected. With an average current density of 35 mA $m^{-2}$ (day 45-55, Figure 3.2b), it can be calculated that about 0.5 mmol of protons are produced per day. This amount of protons produced locally at the anode can indeed cause local acidification. The results indicate however that local buffer capacity, in companion with proton diffusion to the cathode, were able to prevent bulk acidification in the studied microcosms. Lowering the resistance in the external circuit from 500 Ω to 100 Ω did not result in a higher current. This is in conjunction with the observations of Helder et al. on an a rooftop MFC (Helder et al., 2013b). Although they were not able to indicate a cause for this phenomena, from this work it could be observed that it was mainly due to a deterioration of cathode performance. The low cathode potentials were most likely caused by an accumulation of organic detritus (e.g. degraded leaves, algae, roots) on the cathode electrode, leading to a low $O_2$ availability for electrochemical reduction.

Overall, a sequence of steps can be distinguished; initially, during early waterlogging conditions and plant development, a high redox potential due to the presence of $O_2$ and other soluble electron acceptors is noticed. Gradually organic carbon becomes available in the rhizosphere, electron acceptors are being depleted except for the electrode and redox potential decreases leading to current generation. Over time, more organic carbon becomes available than the current generating bacteria can metabolise and $CH_4$ production starts and cannot be stopped anymore.

## 4.3 Archaeal communities in the rhizosphere of plant-MFCs

Few microbial communities of anodes of Plant-MFCs have been elucidated (Kaku et al., 2008; Cabezas De Rosa, 2010; De Schamphelaire et al., 2010b; Timmers et al., 2012b). Of the microbial communities studied, focus has been placed on the anode bacterial community and not on the archaeal microbial community. In the current study methanogenic community slightly dominated by $H_2$-dependent methanogens was detected. This was also the case in several other studies and sometimes even more pronounced in closed circuit compared to open circuit conditions. De Schamphelaire et al. (2010b) have seen a relative enrichment of $H_2$ utilizing methanogens in Plant-MFCs consisting of a soil or vermiculite rhizosphere with graphite felt electrodes, which only covered a small part of the rhizosphere. Timmers et al., (2012a) characterized the microbial populations in the anode of a high current (167 ± 65 mA $m^{-2}$) vs. a low current (67 ± 58 mA $m^{-2}$) producing Plant-MFC (*Glyceria maxima*) with the complete anode compartment filled with electrode material. The relatively high current indicated that in that case sufficient organic substrate was present in the anode under reducing conditions. In their study, a selection towards a $H_2$-based metabolism was detected after 225 days of operation where 95% of the archaea could be related to the genus *Methanobacterium* in the high current producing Plant-MFC vs. a more diverse archaeal community in the low current MFC. The bacterial community supported these results as $H_2$ producing clostridia were abundantly found in the high current producing systems (Timmers et al., 2012b).

Using a sediment MFC, Cabezas da Rosa (2010) found that $H_2$-based methanogenesis was more important compared to acetate based methanogenesis at the archaeal community level, i.e. *Methanosaetaceae* were less abundant compared to *Methanosarcinaceae* and *Methanomicrobiaceae* in the current generating systems. This result was corroborated by the stable isotope ratios of $CH_4$ indicating lower acetoclastic $CH_4$ production in closed circuit systems (Cabezas De Rosa, 2010).

In the microcosms studied in this work, an alternative electron acceptor was introduced in the rhizosphere aimed at lowering the activity of the methanogenic microbial community. As seen in other (plant) MFC based anodes, also here hydrogenotrophic methanogens were most abundantly detected. This indicates that to effectively lower methane emissions by introducing an anode, the focus should be on stimulating hydrogen to current conversions.

## 4.4 Outlook & challenges

From this longitudinal study several challenges for the control of methane emissions from rice paddies or other wetlands on a larger than lab-scale by means of a bioelectrochemical system can be envisioned. Condensing the limited information that is known on (competitive) inhibition of $CH_4$ formation in anodes of (sediment) bioelectrochemical systems, it shows that contradicting data are present in the existing literature, because of 1) different inocula, 2) a varying electrode configuration, 3) a varying organic loading rate and 4) a different electrochemical control (Freguia et al., 2008; Ishii et al., 2008; Kaku et al., 2008).

A first challenge is the effective range of the anode and the technical application of the bioelectrochemical system. In order to achieve an effect, carbon granules were applied in a 2/3 ratio vs. the amount of vermiculite matrix. Combining these dispersed granules with a suitable current collector and a reference system poses a great challenge. On top of that, agricultural practices (e.g.

ploughing) can interfere with the complete bioelectrochemical system (including reference electrode, cathode and electrical control). A solution to this challenge might be the use of electron shuttle molecules (phenazines, humic substances etc). However the effectiveness of these molecules in electron transfer processes in soils and sediments needs to be established. Moreover, these molecules can also interfere with other processes than electron transfer.

The second challenge is the question of electrochemical control. In this work, electrochemical control was applied by using an external resistor coupled to an $O_2$-reducing (bio)cathode. Visual inspection, polarization curves and electrochemical monitoring of the cathode potential indicated deterioration of cathode performance over time. A probable cause for this deterioration is the amount of organic carbon that accumulated on the cathode electrode over time due to growth of algae and dead (plant)biomass. Moreover, cell performance was highly dependent on the cathodic circadian rhythm. This was most likely due to the variation in $O_2$ concentration at the cathode as in this work temperature and pH were rather stable. Dependence of an oxygen reducing cathode on O2 mass transfer and thus the circadian rhythm has been shown in dedicated studies (Strik et al., 2010; Ter Heijne et al., 2010a). This is something that needs to be adequately addressed before stable field performance can be ensured. Another option for electrochemical control, is the polarization of the anode electrode at a certain potential (e.g. 0 vs. SHE). This might however lead to large energy investments due to the internal resistance of a sediment-BES configuration (see Supplementary information S3.2, Figure S3.1).

A third challenge is the durability of $CH_4$ emissions mitigation over a long period of time. In this work, it was established that $CH_4$ emissions could be postponed by introducing a bioanode. However, the effectiveness on a time scale longer than one growing season needs to be determined. A longer effective working period can possibly be obtained in combination with an anode of conductive biochar in the rhizosphere. Biochar is able to increase the abundance of methanotrophic bacteria (Feng et al., 2012). During an anaerobic period, a conductive biochar can act as an anode, while during an aerobic period it can act as a methanotrophic stimulant. The application of agricultural practices such as mid-season drainage (Xu et al., 2007) to provide a competitive advantage for the current generating microbial community (that is able to withstand $O_2$ (Freguia et al., 2008; Nevin et al., 2011a)) in conjunction with methanotropic stimulation is another option.

In conclusion, It was shown that placing an anode of a bioelectrochemical system in a waterlogged rhizosphere led to current generation before methane emissions started, possible leading to postponement of methane emissions from these anoxic systems in the short term. However, in the long-term (i.e. period of complete rice cropping season), low methane emissions could not be maintained due to an excess of organic matter in the rhizosphere. Based on qPCR analysis of the archaeal community in the rhizosphere/anode plane, $H_2$ was indicated as the most important precursor for methane production. This shows that there was an effective competition on the level of acetoclastic methanogenesis, but that the competition for $H_2$ needs to be enhanced to achieve a more effective and sustainable mitigation of methane emissions by means of a bioelectrochemical system. The effect of local acidification due to anodic metabolism on methanogenic metabolism could not be established in this work. To achieve a practical, large scale application of a bioelectrochemical system to mitigate methane emissions, a combined strategy with other methane mitigation approaches is envisioned.

## Acknowledgements

J.A. was funded by the European Community's Seventh Framework Programme FP7/2007-2013 under Grant Agreement No. 226532. W.V. is supported by a Ghent University Multidisciplinary Research Partnership (MRP): Biotechnology for a sustainable economy (01 MRA 510W). The authors thank Jan Vermeulen for assistance with trace gas analysis. Simon Decorte, Joachim Desloover and Giovanny Ganendra are acknowledged for their fruitful discussions on the manuscript.

## Supplementary information:

### S1. Example calculation to convert current density, chemical oxygen demand removal rate and current in the same units

Electrical current density (A $m^{-2}$), chemical oxygen demand removal rate (gCOD $d^{-1}$) and methane emissions (mg$CH_4$ $m^{-2}$ $h^{-1}$) can all be recalculated into the same units. All three parameters express, in essence, the movement of electrons. Here some example calculations are presented to clarify these conversions.

Electrical current density J (A $m^{-2}$), can be expressed as the amount of charge Q, expressed in coulomb (C) per area A ($m^2$) per time t (s)

$Q/(t*A) = J$ (A $m^{-2}$) (Eq. S3.1)

The amount of charge can be expressed as mole of electrons by conversion with the Faraday constant (96485 C mol $(e^-)^{-1}$). Thus electrical current density is a measure of the amount of charge that moves in a given time through a certain area.

Organic matter removal can be expressed as chemical oxygen demand (COD) removal per day. This can also be expressed as the movement of charge in a given time. For example acetate oxidation with $O_2$ can be written according to two half reactions, the first is the oxidation of acetate and the second the reduction of $O_2$ to water.

$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$ (Eq. S3.2)

$2O_2 + 8e^- + 8H^+ \rightarrow 2H_2O$ (Eq. S3.3)

From these two half reactions it can be established that 1.1 g of $O_2$ (32 g $mol^{-1}$) is needed for the oxidation of 1 g of acetate (59 g $mol^{-1}$). Moreover, as can be seen in S2 and S3, electrons are being displaced. Accordingly, the oxidation of organic matter per area per time can be expressed as a current density. For example oxidizing 1 g organic matter per $m^2$ per h results in a current density of 3.4 A $m^{-2}$.

1 (gCOD $m^{-2}$)/32 (g $mol^{-1}$ $O_2$) * 4 (mol $(e^-)$/$mol^{-1}$ $O_2$) *1/3600 (h $s^{-1}$)*96485 (C mol $(e^-)^{-1}$) = 3.4 A $m^{-2}$

Methane removal or production can also be expressed as a current density when applying the same reasoning.

$1HCO_3^- + 9H^+ + 8e^- \rightarrow 1CH_4 + 3H_2O$ (Eq. S3.4)

For example producing 10 mg$CH_4$ per $m^2$ per h results in an equivalent current density of 0.13 A $m^{-2}$

$10*10^{-3}$ (g$CH_4$ $m^{-2}$)/16 (g $mol^{-1}$ $CH_4$) * 8 (mol $(e^-)$ $mol^{-1}$ $CH_4$) *1/3600 (h $s^{-1}$)*96485 (C mol $(e^-)^{-1}$) = 0.13 A $m^{-2}$

Conversely, current density can be expressed in equivalent units such as gCOD $d^{-1}$ or mg $CH_4$ $m^{-2}$ $h^{-1}$ enabling fast comparison of the various metablic rates. See also Arends et al. (2012).

## S2. Estimation of electrical operating costs for a poised anode in a wetland

To estimate the amount of electrical power and thus the electrical operating cost per $m^2$ of plant-MFC in case of anode potentiostatic control, several parameters influence the outcome of this estimation. One important parameter is the distance between the reference electrode and the anode electrode. This causes an ohmic voltage drop that must be compensated for to achieve a good potentiostatic control of the anode. This ohmic voltage drop is dependent on the current that flows through the system (this can be understood as the methane mitigation rate) and the resistivity of the matrix (in this case rice or wetland soil). Here an estimation is made for the following case, a wetland soil with 1 reference electrode, a maximum effective anode-reference distance of 1 m and an energy price of € 0.20 $kWh^{-1}$. Soil resistivity was varied between 0 and 50 Ω m (Domínguez-Garay et al., 2013) and current density between 10 and 300 mA $m^{-2}$ which is equivalent to 0.13-3.9 $mgCH_4\ m^{-2}\ h^{-1}$ (Eq. S4). Within these parameters 55 scenarios were calculated which are depicted in Figure S1. From this estimation it can be seen that when aiming for an appreciable methane mitigation rate, a considerable monthly cost is incurred. Options to reduce this cost are placing more reference electrodes (extra capital cost) or opting for less sophisticated electrochemical control by using an external resistance.

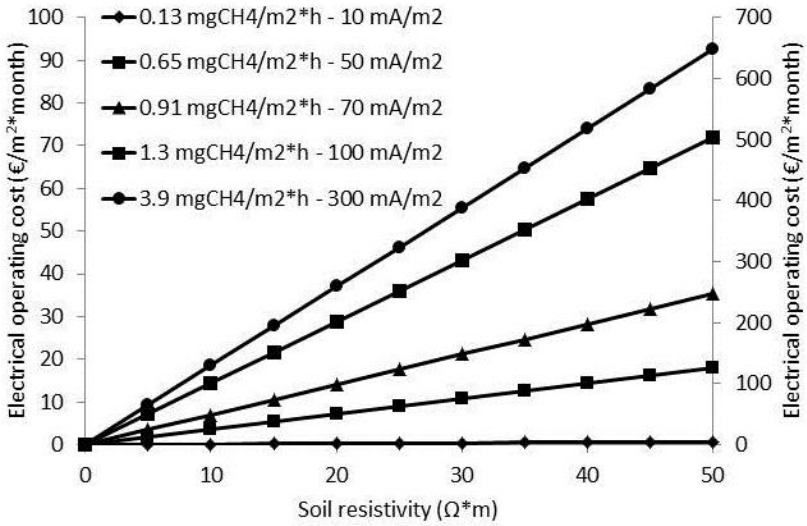



***Figure S3.1:*** *Estimation of electrical operating costs for a poised anode in a wetland based on soil resistivity and current density. Scenario of 3.9 mg $CH_4\ m^{-2}\ h^{-1}$ – 300 mA $m^{-2}$ is depicted on the right axis.*

Other capital costs such as anode and cathode materials, electrical wiring and control periphirals have already been described elsewhere for BES (Rozendal et al., 2008b; Foley et al., 2010). On the profit side a small amount of electrical power can be noted (when using MFC mode) and one can possibly enter in a $CO_2$-certificate trading scheme when emissions are succesfully lowered.

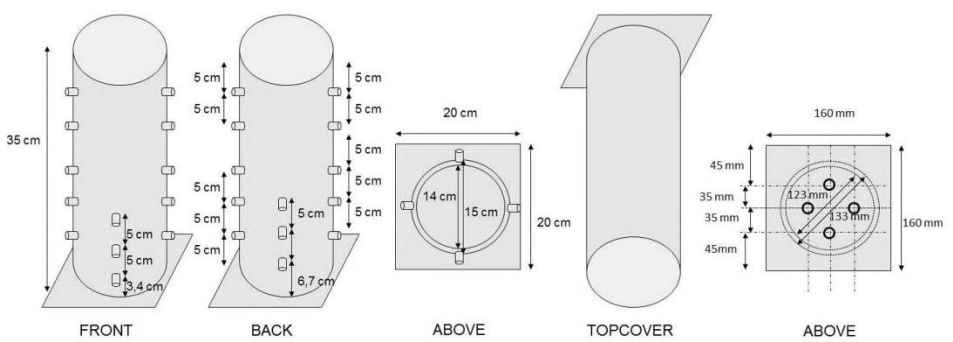



***Figure S3.2:*** *Left 3 schematics: bottom part of the microcosm used for rice growth. The top ledge contained a 2 cm wide rim (not shown) with a 1 cm wide slot to accommodate the closed chamber for headspace gas measurements. Right 2 schematics: Chamber to allow headspace gas measurements on the microcosms.*

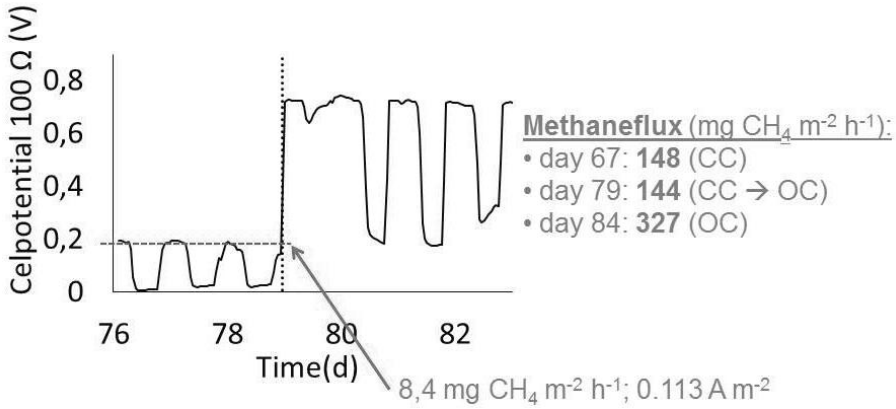



***Figure S3.3:*** *Result from the exploratory study where removing the external resistance resulted in an increased $CH_4$-flux 22 times higher than can be estimated based on the current in closed circuit. CC: Closed Circuit, OC: Open Circuit.*

***Table S3.1:*** *Overview of all experiments.*

| # | # of plants | Seedling age at transplanting (week) | Added organic carbon (kg $m^{-2}$)[1)] | OC/CC | Inoculum (A and/or M) | Granules in anode [2)] | Plant growth area ($m^2$) |
|---|---|---|---|---|---|---|---|
| Exploratory study | | | | | | | |
| E1 | 2 | 7 | 2 | OC | A & M | Y | 0.015 |
| E2 | 2 | 7 | 2 | CC | A & M | Y | 0.015 |
| E3 | 2 | 7 | 2 | CC | A & M | No [3)] | 0.015 |
| E4 | -[4)] | - | 2 | OC | A & M | Y | 0.015 |
| E5 | - | - | 2 | CC | A & M | Y | 0.015 |
| E6 | 2 | 12 | 2 | OC | A & M | Y | 0.032 |
| E7 | 2 | 12 | 2 | CC | A & M | Y | 0.032 |
| E8 | 2 | 12 | No | CC | A & M | Y | 0.032 |
| E9 | - | - | 2 | CC | A & M | Y | 0.032 |
| E10 | - | - | 2 | CC | A | Y | 0.032 |
| Detailed study | | | (kg $m^{-2}$ $d)^1$ | | | | |
| M1 | 2 | 3 | No | CC | A | Y | 0.015 |
| M2 | 2 | 3 | No[5)] | CC | A | Y | 0.015 |
| M3 | 2 | 3 | 0.013 | CC | A | Y | 0.015 |
| M4 | 2 | 3 | 0.013 | CC | A | Y | 0.015 |

1) Composition see main text.
2) amount of granules based on previous work (Arends et al., 2012)
3) only current collectors present in the anode compartment.
4) non-planted systems are considered as sediment-MFC
5) liquid was added here without organic carbon.
OC: open circuit.
CC: closed circuit over 500 Ω external resistance.
A: anodic effluent of a well performing MFC in the lab 10 vol-% total anode compartment.
M: anaerobic methanogenic sludge from WWTP 'Ossemeersen' Gent, Belgium 1 vol-% of total anode compartment.

***Table S3.2:*** *Quality control parameters for qPCR analysis. Parameters as obtained during analysis with StepOnePlus software V2.2.2. Detection limit calculated to copies gram$^{-1}$ wet weight of the sample taking dilution and extraction efficiency into account.*

| | Amplification plot | | Standard Curve | | | |
|---|---|---|---|---|---|---|
| | Threshold (ΔRn) | Baseline | Slope | $R^2$ | Eff (%) | Detection limit (copies $g^{-1}$) |
| Total Bacteria | 3.1 | 3 | -3.3 | 0.98 | 100 | $3.1x10^5$ |
| Total Archaea | 4.5 | 3 | -3.2 | 0.97 | 100 | $4.4\ x10^3$ |
| *Methanobacteriales* | 2.9 | 3 | -3.6 | 0.99 | 88 | $3.9\ x10^3$ |
| *Methanomicrobiales* | 2.6 | 3 | -4.0 | 0.99 | 78 | $3.6\ x10^3$ |
| *Methanosarcinaceae* | 4.5 | 3 | -3.7 | 1.00 | 86 | $4.8\ x10^3$ |
| *Methanosaetaceae* | 2.6 | 3 | -3.6 | 0.99 | 91 | $5.1\ x10^3$ |

# CHAPTER 4: THE USE OF A DRAIN TUBE CATHODE IN A SEDIMENT MICROBIAL FUEL CELL LOWERS INTERNAL RESISTANCE BUT REDUCES POWER OUTPUT

This chapter has been redrafted after:

Arends, J.B.A., D'haese, A., Verstraete, W. 2011. Cathodes for sediment microbial fuel cells. Presented as a poster at: 1st International PlantPower conference, February 2011, Gent, Belgium and 3rd International MFC Conference, june 2011, Leeuwarden, The Netherlands.

## Abstract

Sediment microbial fuel cells are a form of bio electrochemical technology which can be envisioned to have a commercial application i.e. for powering remote sensing equipment. To improve the range of possible applications, the power and current output needs to increase. This can be done by increasing current and power densities or by increasing the surface area. Increasing surface area leads to disruption of the natural environment due to digging activity upon installing the anode. Moreover, the cathode is prone to clogging and fouling when placed in a surface water. In this work a tubular cathode was placed in the sediment and its performance compared to a top placed cathode. A membrane tube showed the highest and most sustainable current (87 ± 2.5 mA $m^{-2}$) and power density (38 ± 2.1 mW $m^{-2}$). A drain tube cathode wrapped with geo-textile showed decreased performance in terms of current (47 ± 21.5 mA $m^{-2}$) and power (14 ± 8.8 mW $m^{-2}$) but also a lower overall internal resistance (1.1 Ω $m^2$ vs. 2.6 Ω $m^2$). Cathodes placed in the overlying water layers showed in general a poor performance in comparison with the bottom cathodes. A tubular cathode can be extended as a membrane electrode assembly and deployed by means of horizontal drilling over larger surface areas.




## 1. Introduction

Sediment microbial fuel cells (SMFC) have thus far been the type of bioelectrochemical systems (BES), closest in development to an application. Their applicability lies in producing power to drive measuring devices typically in remote areas. Examples include meteorological buoys (Tender et al., 2008) and other sensory equipment (Carapezza et al., 2008; Donovan et al., 2008; Thomas et al., 2013).

Thus far, the design of these systems has been straightforward. The anode is buried in the sediment where reduced organic material or sulphides are present which can be (a)biotically oxidized to provide reducing power. The cathode is usually a (biological) oxygen-reducing cathode suspended in or floating on top of the above water layer (Carapezza et al., 2008; Donovan et al., 2008; Tender et al., 2008; Takanezawa et al., 2010; Thomas et al., 2013).

The power output and current density of a sediment microbial fuel cell is usually low, in the order of 50-100 mW $m^{-2}$ and up to some 200 mA $m^{-2}$. This low power and current density can be attributed to limited substrate availability (He et al., 2007; Scott et al., 2008b), (in)efficient catalysis at the electrodes and a high internal resistance of the system. For BES reactor systems, various measures have been investigated regarding their effectiveness in reducing the internal resistance of the system. These include: improving anolyte and catholyte conductivity and buffering capacity (De Schamphelaire et al., 2010a; Sleutels et al., 2010), electrode spacing (Cheng et al., 2006), electrode size ratio (Oh & Logan, 2006), the use of spacers to improve liquid flow (Sleutels et al., 2011) and the use of various types of membranes (Kim et al., 2007; Sleutels et al., 2009b).

Using sediment based systems, there are far less possibilities to engineer these systems for increased power output. There is no membrane and by virtue of that, electrode spacing needs to be sufficient in order to avoid crossover of reactants from anode to cathode and vice versa (He et al., 2007). Increasing conductivity is also an unlikely approach since in natural systems where plants grow conductivity is in the order of 5 mS $cm^{-1}$. An exception to that is conductivity in brackish or saline environments where conductivities can be up to 10 times higher (De Schamphelaire et al., 2010a).

The parameters that can be reasonably engineered in sediment systems is electrode size and spacing and making clever use of natural convection of the water layer (Nielsen et al., 2007). In this work, a new cathode design is evaluated where the cathode is placed in the sediment layer close to the anode in order to achieve a lower internal resistance and thus create an opportunity for a higher power output and current density. For larger wetland areas, it is unpractical and expensive to bury large lengths of ion exchange membranes in the sediment. Therefore the suitability of a drain tube wrapped in geotextile is evaluated as a replacement for ion exchange membranes. This design can be introduced in the sediment by means of horizontal drilling (Timmers et al., 2013).

## 2. Materials and methods



### 2.1 MFC construction

A sediment MFC consisted of a container with dimensions 35.3*12.7*26 cm (l*w*h) (Figure 4.1). On the bottom of the container the cathode was placed surrounded by graphite granules (1,5-5 mm, Mersen) (Clauwaert et al., 2007b). On top of the granules, washed beach sand containing no organic carbon was placed followed by a water layer. $MFC_{drain}$ contained a cathode consisting of a drain tube (outer dimensions: d = 6,5 cm, L=30 cm) wrapped with geotextile (Deschacht plastics, Belgium). $MFC_{mem}$ had a cathode consisting of a tubular cation exchange membrane (Ultrex CMI-700, Membranes International, Inc., USA)(Clauwaert et al., 2007b) of the same inner dimensions. Both cathodes were filled with 270 g dry weight graphite granules. The ends of the cathodes were sealed and connections for liquid flow were provided. Current in the anode was collected with a carbon felt (25*10*0.33 cm; Alfa Aesar, Germany) interwoven with graphite rod (d = 5 mm, Morgan, Belgium). The cathode current collector was a similar graphite rod. The graphite rods were electrically connected to a fixed external resistance during experiments by means of an insulated copper wire. A watertight electrical connection between the wire and the rod was established by using conductive carbon cement (CCC, Leit-C; Fluka) and vulcanizing tape. Later in the experiment, a carbon felt cathode (similar to the anode current collector) was added on top of the standing water layer in both MFCs and connected via a separate resistor to the anode electrode. To be able to measure pore liquid organic carbon, a rhizon sampler was located between the anode granules (10 cm porous, Rhizosphere research products, The Netherlands).

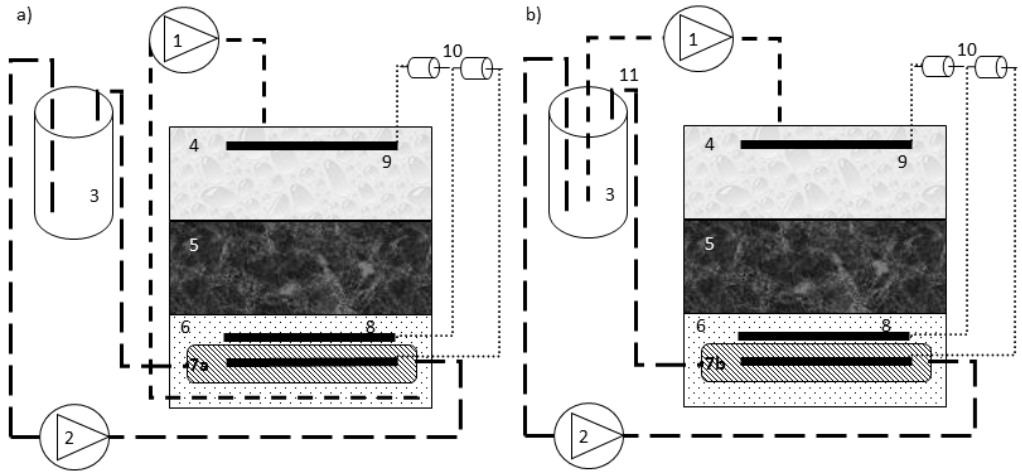



***Figure 4.1:*** *Schematic overview of the membrane tube cathode (a) and draintube cathode (b). 1) anode recirculation pump, 2) cathode recirculation pump, 3) recirculation vessel, 4) standing water layer, 5) sand layer, 6) anode granules, 7a) membrane tube cathode, 7b) drain tube cathode, 8) anode current collector, 9) top cathode, 10) external resistor and electrical connections, 11) level control.*

## 2.2 MFC operation

Both MFCs had a differing hydraulic recirculation scheme. This is due to the fact that $MFC_{drain}$, containing a drain tube cathode, had no hydraulic separation of anode and cathode liquids while $MFC_{mem}$ had a hydraulic separation of anolyte and catholyte due to the membrane. Both MFCs were supplied with tapwater containing COD from the top (an equal mixture of starch and acetate equivalent to 20 ton dry matter deposition $ha^{-1}$ $yr^{-1}$). The effluent of $MFC_{drain}$ came out via the drain tube, through a water lock to maintain the same standing water level, into a central vessel where it was re-oxygenated. Part of the liquid was recirculated to the top (anolyte) while part of it acted as the aerated catholyte. The anode effluent of $MFC_{mem}$ came out via a perforated tube at the bottom of the container through a water lock to maintain the same standing water level. The catholyte was recirculated over an aerated external vessel through the membrane tube cathode. Flow rate in the cathode was 1.1 ± 0.1 l $h^{-1}$ leading to an effective HRT of 11 ± 1.2 min. based on cathode liquid volume. Experiments were performed at room temperature 20 ± 2 °C. To achieve an electroactive microbial community in both the anode and the cathode compartment of the sediment MFC, the anodes and cathodes were inoculated with 200 ml effluent from a well performing MFC present in the lab (Clauwaert et al., 2007b).

## 2.3 Analytical techniques, calculations and data representation

Chemical oxygen demand (COD) was determined using commercial kits according to the manufacturer's instructions (Nanocolor® COD, Machery-Nagel, Germany). Cell potential over the external resistance (1000 Ω for start-up periods, 500 Ω for continuous operation) and anode potential vs. a reference electrode (Ag/AgCl, BASi, United Kingdom) were continuously logged (Data acquisition unit HP39470A, Agilent technologies). The reference electrodes were regularly monitored versus a calomel electrode (+244 mV vs. Standard Hydrogen Electrode (SHE); QIS, the Netherlands). All electrode potentials are reported versus SHE potential. Polarization curves were recorded with a potentiostat (Bistat, Biologic, France) at a scan rate of 1 $mVs^{-1}$ following a 15 min stabilization period

in open circuit. The polarization curve was followed by a 15 min. stabilization period at a cell potential of 200 mV after which the current interrupt technique was applied. Data were recorded for every 10 ms after the current was interrupted. The first data point was used for the calculation of the internal resistance. Internal resistance was calculated according to Liang et al. (2007). During polarization, only a top or a bottom cathode was connected. Hourly averages for current and power density are reported normalized to the projected surface area of the respective cathode.

## 3. Results & Discussion

### 3.1 Membrane vs. drain tube cathode performance in the sediment



The two MFCs were acclimatized for 40 days to allow the microbial community to achieve a stable performance. During the measurement period after it was clearly seen that the feeding pattern could be distinguished in the output of the $MFC_{drain}$ (Figure 4.2). The anode potential was affected more than the cathode potential as upon addition it decreased within an hour by about 0.5 V. The cathode potential decreased only 0.15 V within the same timeframe. This indicates that oxygen transfer from the cathode through the drain tube towards the anode was the most disrupting process as opposed to transfer of organic matter from the anode towards the cathode. In other words, organic carbon supply to the anode was a limiting factor in the $MFC_{drain}$. No effect of organic matter addition could be seen in the potentials of the $MFC_{mem}$ (Figure 4.2a,b). Although the cell potentials differed considerably over time, average current densities were rather similar i.e. 53 ± 17 mA $m^{-2}$ and 57 ± 2.0 mA $m^{-2}$ for the $MFC_{drain}$ and the $MFC_{mem}$ respectively.

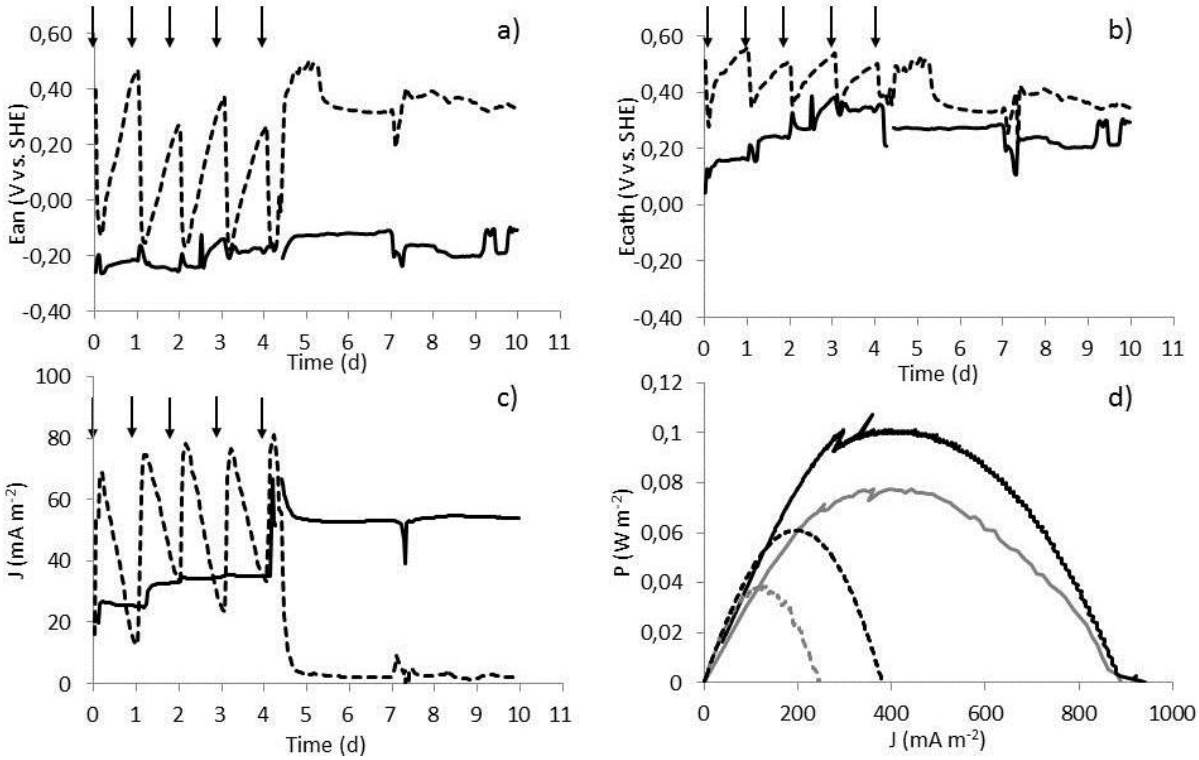



***Figure 4.2:*** *Anode (a) and cathode (b) potentials and current density (c) of $MFC_{mem}$ (solid line) and $MFC_{drain}$ (dashed line) over a 10 day period. Arrows: organic carbon addition. Power curves (d) recorded at day 1 (black line) and 8 (grey line).*

Overall recovery of COD as current was also similar between both reactors. For the $MFC_{drain}$, 2.2 % of the daily added COD was recovered over a 24 hour feeding cycle (4 cycles). The $MFC_{mem}$ showed a COD recovery as current of 2.3 %. Accumulation of COD was observed in the $MFC_{mem}$ suggesting that this reactor was less efficient in the removal of organic matter. COD concentrations were 7.5 times as high in the $MFC_{mem}$ compared to the $MFC_{drain}$ (460 mg $L^{-1}$ vs. 60 mg $L^{-1}$). Unlike in chapter 2 and 3, in this setup limited other electron acceptors were present that could have been used to metabolize the organic carbon as tapwater was the water source. The above results of potentials and COD removal suggest that there was an efficient transfer of $O_2$ from the tubular cathode to the anode in the case of the $MFC_{drain}$ whereas in the $MFC_{mem}$ little $O_2$ transfer via the membrane occurred. The results also indicate that the transfer of organic matter from the anode towards the cathode in the $MFC_{drain}$ did not impact cathode performance to a large extent. The effect of substrate crossover was clearly visible when addition of organic carbon stopped. The current density of the $MFC_{drain}$ dropped to almost 0 mA $m^{-2}$, indicating no potential difference between anode and cathode. The current density of the $MFC_{mem}$ was able to increase upon decreasing the external resistance on day 4.5 (Figure 4.2c)



Power curves revealed the same performance trend, where the $MFC_{mem}$ achieved a maximum power and current density of 101 mW $m^{-2}$ and 941 mA $m^{-2}$ whereas the maximum performance during this period of the $MFC_{drain}$ was 61 mW $m^{-2}$ and 380 mA $m^{-2}$ (Figure 4.2d).

## 3.2 Top versus bottom cathode

To understand how the internal resistance is affected by placing the cathode in the sediment, another cathode was placed on top of the water layer. Little current flowed through the cathode that floated on top of the $MFC_{mem}$ (Figure 4.3, stage 1). Switching the top cathode of $MFC_{mem}$ with the top cathode of the $MFC_{drain}$ did not improve the performance (Figure 4.3, stage 2), indicating that the bottom cathode of $MFC_{mem}$ had a stronger catalytic activity. This might be due to the longer acclimation period (70 days extra) used for the bottom cathodes which allowed an oxygen reducing biocathode to develop. The top cathode of $MFC_{drain}$ had a lower performance compared to the bottom cathode in the first stage. Switching the top cathode of $MFCd_{rain}$ with the top cathode of $MFC_{mem}$ did not result in a change in performance for the top cathode, i.e. a lower performance compared to the bottom cathode. Switching back to the original top cathodes gave a poor performance, similar to the topcathode in $MFC_{mem}$ (Figure 4.3, stage 3). Indicating that current rather flowed through the bottom cathode compared to the top cathode.

These results show that the performance of a top cathode compared to a bottom cathode are influenced by the type and activity of the bottom cathode. In case a membrane tube bottom cathode was used, which had limited influence of incoming organic carbon, top cathode performance was poor. In case a drain tube bottom cathode was used, the top cathode was able to draw current, possibly due to organic carbon interfering with the performance of the bottom cathode (Figure 4.3).

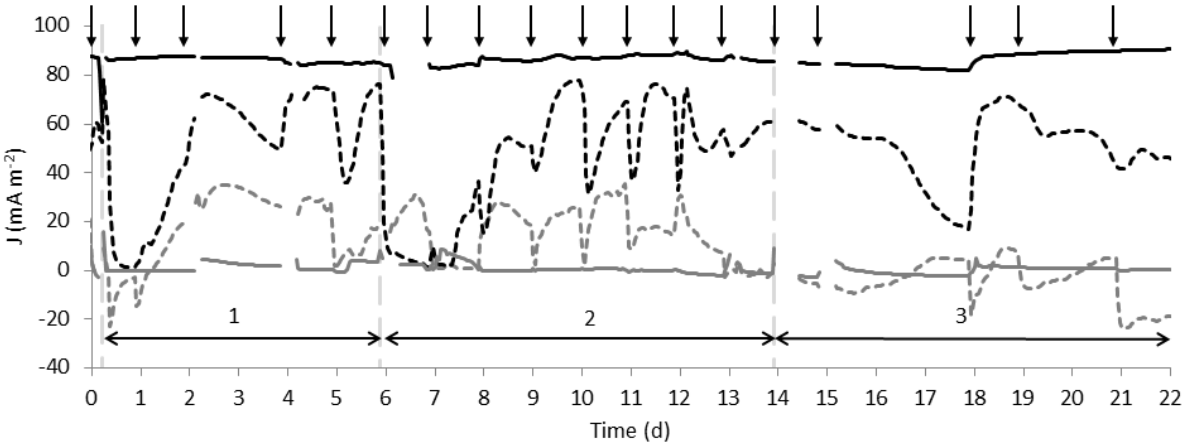



***Figure 4.3:*** *Current density obtained by the four cathodes, $MFC_{mem}$ (solid line) and $MFC_{drain}$ (dashed line), bottom cathode (black line) and top cathode (grey line). Vertical lines: top cathode exchange, 1) top cathodes at their original position. 2) top cathode $MFC_{drain}$ on $MFC_{mem}$ and vice versa, 3) top cathodes back at their original positions. Arrows: organic carbon addition. Gaps in current data are due to polarization curves or other manipulations.*

Several techniques exist to determine the internal resistance of a BES such as electrochemical impedance spectroscopy, polarization curves and current interrupt. According to Liang et al. (2007), subtracting the value of the current interrupt technique (ohmic resistance) from the slope of the polarization curve for the individual electrodes will give the value of the specific resistance of the electrode. Applying this reasoning to the set-up used here, the ohmic resistance showed to be lowest for the cathodes placed in the bottom with the $MFC_{mem}$ having the lowest cathode ohmic resistance (Figure 4.4). The anode resistance, including activation overpotential, is in all cases lower than the cathode resistance. Interestingly, the cathode resistance is higher for the $MFC_{mem}$ compared to $MFC_{drain}$ but also the cathode resistance is higher for the bottom electrodes compared to the top electrodes. This is contradicting the current and power output data recorded in the polarization curve and during the long term operation. Considering that no reference electrode could be placed in the cathode compartment, the large cathode resistance can also be caused by 'membrane/separator' polarization and transport effects.

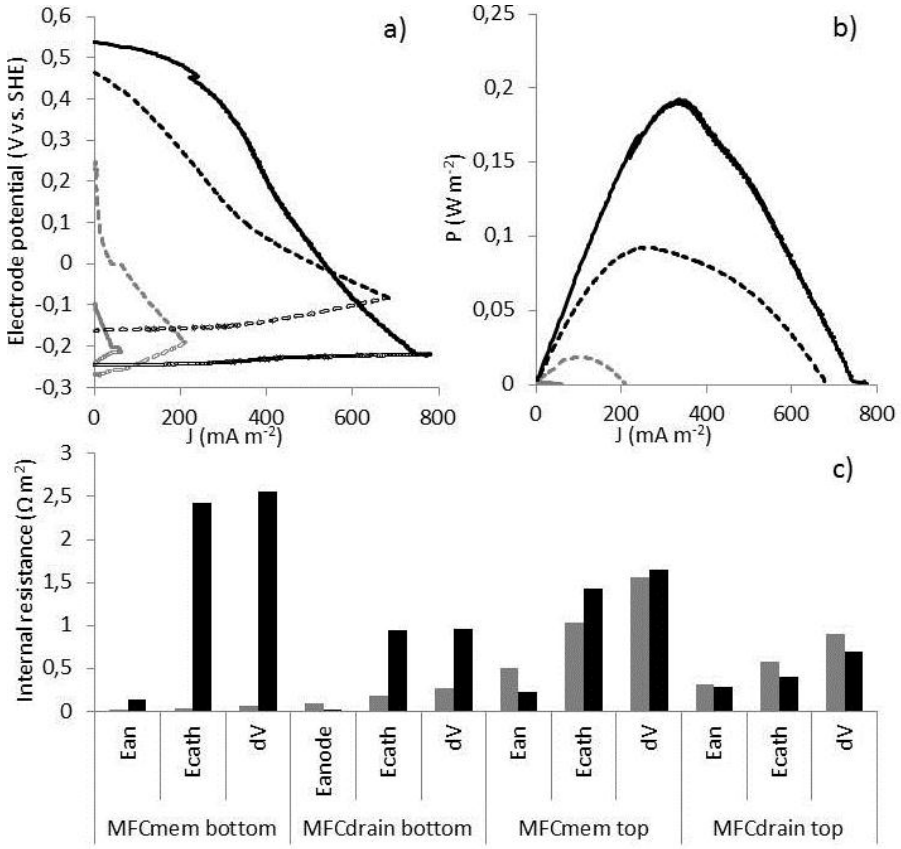



***Figure 4.4:*** *Polarization curve (a), power curve (b) and distribution of internal resistance (c) of $MFC_{mem}$ (solid line) and $MFC_{drain}$ (dashed line), bottom cathode (black line) and top cathode (grey line). In (a), open lines are anode potentials, solid lines represent cathode potentials. In (c) black bars represent electrode resistance, grey bars represent ohmic resistance. Resistances calculated according to Liang et al. (2007). Cathode resistance includes also the influence of the membrane/separator.*

Cathode functioning and placement are key parameters in sediment MFCs. In this work, a new configuration with a drain tube cathode wrapped in geo-textile buried in the sediment was introduced. This was compared with a similar sized cathode of cation exchange membrane and a conventional cathode in the water layer. Most of the internal resistance in a tubular plant MFC design could be attributed to the anode and membrane compartment (Timmers et al., 2013). The differing results presented here can be explained by their use of non-renewable electron acceptor, ferricyanide, which is known for good electron transfer at an electrode while in this work $O_2$ was used as the final electron acceptor. Oxygen supply to the buried cathode is still an engineering challenge. Assuming a current density of 40 mA $m^{-2}$ (Figure 4.2c and 4.3), 0.6 $gO_2$ $d^{-1}$ $m^{-2}$ is needed to sustain this current density. Taking a dissolved oxygen concentration of 8 mg $L^{-1}$, a flow rate of 3 L $h^{-1}$ $m^{-2}$ is needed to supply the electron acceptor i.e. some 25 times less as used in this setup. It has been proposed to turn the adverse effect of radial oxygen loss by plant roots on the anode (Timmers et al., 2011) into a benefit by using the oxygen as a source of electron acceptor by placing the cathode around the roots. This was a successful strategy for a short time (Takanezawa et al., 2010). The long term benefit of this strategy still needs to be elucidated as the roots are also a source of organic matter (excretions, dead biomass) that will compete for $O_2$ and can possibly clog the cathode.

The design proposed here is in need of some energy input to supply the cathode with oxygenated liquid. Therefore the design can be applied in (constructed) wetlands where an active water flow is present. An alternative is application of the proposed system in greenhouses where oxygenated liquid is pumped already to supply the plant with nutrients. Both applications imply the use in planted areas where anode organic carbon supply can be increased by rhizodeposition processes (De Schamphelaire et al., 2008). Interaction of plant roots with the drain tube needs to be assessed to prevent rupture of the geo-textile. For a practical application of the proposed design with a drain tube buried in the sediment, oxygen supply and consumption over a larger length scale needs to be understood. Next to that, the possible effect of cathode clogging due to aerobic biomass growth needs to be determined over a longer time frame.



## 4. Conclusions

In this work a drain tube wrapped in geo-textile filled with graphite granules was assessed for use as a cathode in sediment microbial fuel cells. A tubular cation exchange membrane filled with graphite granules and a graphite felt placed in the overlying water were the reference designs. A buried tubular cathode had a higher power and current density compared to a cathode in the overlying water layer. The drain tube cathode design resulted in a lowered internal resistance compared to the membranous tube design (1.2 vs. 2.6 $\Omega m^{-2}$). However the drain tube design produced ½ to ⅔ of the current density compared to the membranous cathode. The lower performance was most likely due to substrate crossover. Application of this design is foreseen in (constructed) wetlands or greenhouse horticulture. Upon application of the buried cathode a trade-off needs to be made between a low cost design (drain tube) with lower power output or a high cost design (membrane) with higher power output.

## Acknowledgements

JA and AD'H were supported by a grant from the European Community's Seventh Framework Programme (FP7/2007-2013 under Grant Agreement No. 226532).

# CHAPTER 5: ENHANCED DISINFECTION OF WASTEWATER BY COMBINING WETLAND TREATMENT WITH BIOELECTROCHEMICAL $H_2O_2$ PRODUCTION

This chapter has been redrafted after:

Arends, J.B.A., Vandenhouwe, S., Verstraete, W., Boon N., Rabaey, K. 2013. Enhanced disinfection of wastewater by combining wetland treatment with bioelectrochemical $H_2O_2$ production. *Submitted*

## Abstract

A highly-loaded constructed wetland (up to 44 ± 21 gCOD $m^{-2}$ $d^{-1}$) was linked to a bioelectrochemical system BES to produce hydrogen peroxide for disinfection purposes. The anode delivered a current from the wetland effluent up to 3.5 $Am^{-2}$ (max. 62% anodic efficiency) but was limited in the supply of organic carbon. Hydrogen peroxide production at the cathode was tested at various current densities with a max. rate of 2.7 g $m^{-2}_{electrode}$ $h^{-1}$ in wetland effluent (4 hours at 10 A $m^{-2}$, 41% cathodic efficiency). The resulting hydrogen peroxide (0.1%) was used to disinfect wetland effluent succesfully (<75 CFU $ml^{-1}$ after 1 h contact time). The flow rates can be tuned to produce various water qualities. Thus by linking a BES to a constructed wetland, water treatment with controllable disinfection can be assured in remote locations.

## 1. Introduction

With a growing world population, the pressure on safe (drinking) water supplies increases (Unep, 2008; Un-Habitat, 2010). Numerous technologies exist to provide clean and safe water flows. However, most technologies are only viable at larger scales and/or require maintenance and quality control by trained operators. Therefore it is not possible to supply people in remote areas or in developing countries with adequate high technology (safe) water treatment and supply. Constructed wetlands (CWs) are a basic form of wastewater treatment where (domestic) wastewater is treated in case no access to advanced wastewater treatment facilities is available. Although this seems at first glance a basic form of treatment, this technique is widely applied, in low as well as highly populated areas (Karathanasis et al., 2003; Rousseau et al., 2004; Puigagut et al., 2007). Moreover, wetland water treatment systems are also employed in intensive horticulture systems (Gruyer et al., 2013). Removal of contaminants occurs by the combined action of among others; 1) sorption on bed material and plant roots, 2) microbial transformations and 3) plant uptake (Vymazal, 2005). The main drawback of such a treatment system with little to no operational controls is the (seasonal) variability of organic carbon, nutrient and pathogen removal leading to variable performance. This leads to effluent qualities (in terms of COD or nutrients) that are not always in compliance with regulations (Kern & Idler, 1999; Karathanasis et al., 2003; Puigagut et al., 2007), although various studies also state that wetland treatment confers good removal on some parameters (Rousseau et al., 2008; Gruyer et al., 2013), but all studies are in agreement that microbial indicators are not in agreement with drinking water regulations. Clean water for human consumption will become a more and more scarce commodity in the future due to increased population and higher used per person. Therefore the results of this work were evaluated according to the most stringent regulations. However less stringent applications such as recycling irrigation water in greenhouse horticulture can also be an option for applying the proposed concept.

Besides treatment of wastewaters, (constructed) wetlands have been suggested as a source of electrical power generation by the use of a plant-microbial fuel cell (plant-MFC) (De Schamphelaire et al., 2008; Strik et al., 2008; Yadav et al., 2012). Electrical power is generated based on the subsequent action of plant photosynthesis, root exudation processes and oxidation of organic matter by microorganisms with electron transfer to an anode. Plant organic carbon is excreted by means of various rhizodeposition processes (Neumann & Römheld, 2007). Various microorganisms can, under anaerobic conditions, oxidize this organic carbon and generate electrons that can be transferred to a conducting material, the anode electrode. In this paradigm, the electrons are transferred over an external load to the cathode where oxygen reduction to water takes place. However, plant-MFCs suffer from low performance due to large internal resistances, inconsistent substrate supply and competing reactions (Timmers et al., 2011; Timmers et al., 2012a). This is in contrast with reactor-based MFCs (or bioelectrochemical systems, BES) where a more optimized configuration and feed flow can lead to relative high power densities (Rabaey et al., 2005c; Aelterman et al., 2006b; Logan et al., 2006; Feng et al., 2008; Ahn & Logan, 2010). Interestingly, the cathode reaction can be tuned towards the production of hydrogen peroxide from oxygen, enabling disinfectant production from wastewater (Rozendal et al., 2009; Fu et al., 2010; Modin & Fukushi, 2013).

Here, a combined system (Figure 5.1) is introduced which aims to maximize the benefits of both wetland and BES. In this new concept, part of the wetland will act as a quick filter, retaining and transforming suspended solids into soluble organics. The soluble organics combined with

rhizodeposits will be fed into the anode compartment of a reactor type bioelectrochemical system (BES). The bacteria on the anode will oxidise organic matter into electrical current and $CO_2$. At the cathode $O_2$ is reduced to $H_2O_2$ which can provide downstream disinfection of (at least part of) the effluent of the wetland-BES system. Furthermore year-round operation, also during wintertime, can be achieved as the anode biocatalysts can be adapted to operation at low temperatures whereas plant activity of the wetland can be at a lower level (Jadhav & Ghangrekar, 2009; Patil et al., 2010b; Bergdolt et al., 2013; Helder et al., 2013b). The electron donor for the anode reaction will, during wintertime, not come from active exudation processes, rather from the soluble organics derived from the wastewater and from decaying plant material.

As described above, all four components of this new concept have separately been described in literature namely; 1) the use of constructed wetlands for wastewater treatment, 2) the use of a bioelectrochemical system to directly produce an electrical current from wastewater, 3) the use of a bioelectrochemical system to produce $H_2O_2$ and 4) the use of $H_2O_2$ for disinfection of wastewater (O'sullivan & Tyree, 2007; Labas et al., 2008; Flores et al., 2012; Vargas et al., 2013).



The goal of this work was to conduct an integrated study on the feasibility of the combined wetland-BES concept, focusing on COD removal, $H_2O_2$ production in wetland effluent and disinfection efficiencies of wetland effluent. In other words, 1) can wetland effluent drive current generation and subsequent $H_2O_2$ production in a BES and 2) which fraction of the wetland effluent can be disinfected with this rate of $H_2O_2$ production.

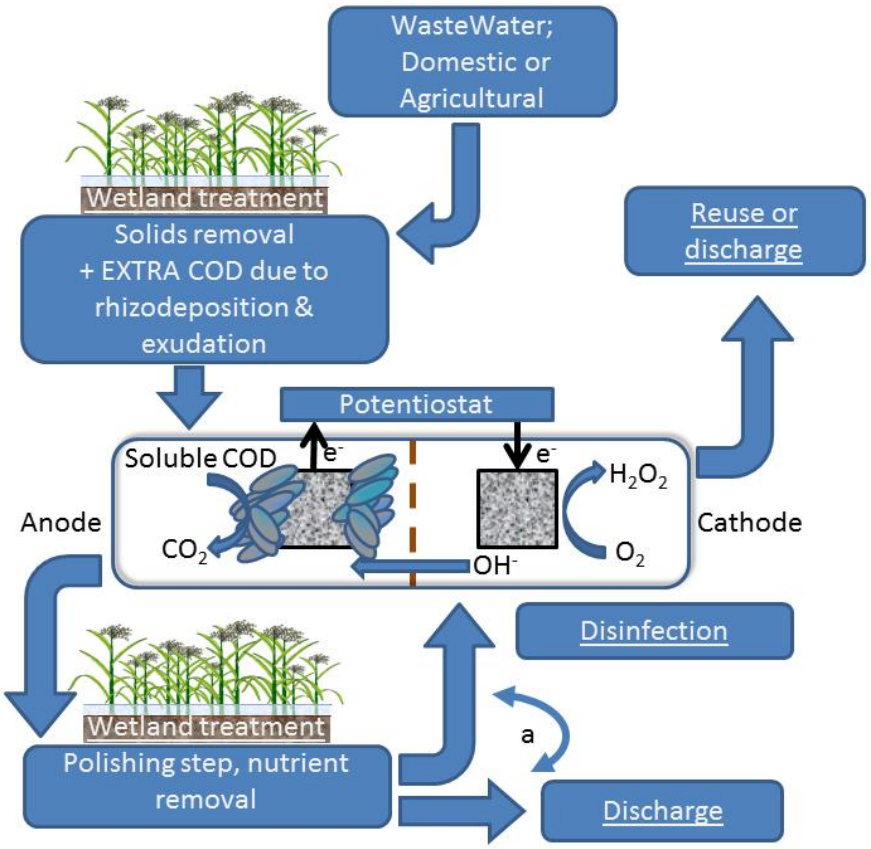



***Figure 5.1:*** *Conceptual overview of wetland wastewater treatment with enhanced disinfection by means of a BES producing hydrogen peroxide. a: flow rate between these two options can be adjusted to meet demand.*

## 2. Materials and Methods

### 2.1 Wetland construction and operation

Two labscale constructed wetlands (58*47*43 cm) were operated in a horizontal subsurface mode in a greenhouse. At the bottom of the container 3 drain tubes (diameter: 6.5 cm) wrapped in geotextile were installed (Deschacht plastics, Belgium). The bed consisted of 10 cm coarse sand (diameter: 0.2-1.6 cm) on top of a layer of 15 cm gravel (diameter: 0.8-2.5 cm) (Figure 5.2). The top layer was planted with sods with an equal amount of freshly developing shoots and rhizomes of common reed (*Phragmatis* spp.) originating from an operational CW (De Pinte, Belgium), leading to a total bed height of ~ 30 cm. The total bed height of gravel and sand is lower compared to most wetlands (60 cm) since the aim is a quick filtration step and collection of soluble COD. Primary wastewater from the domestic wastewater treatment plant of Dendermonde, Belgium and from the hospital Maria Middelares (Gent, Belgium) was collected after screen filtration and stored at 4 °C until it was applied on the wetland. Operation of the wetland started with domestic wastewater but switched to hospital wastewater as the latter contained more COD. In stage 4, sodium acetate was added to increase the soluble organic content of the influent. Removal rates are expressed per $m^2$ wetland surface.



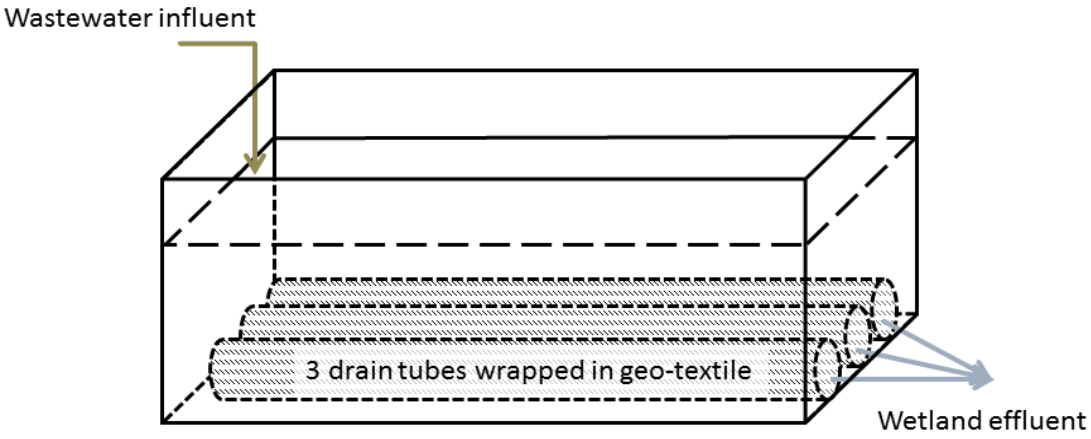



***Figure 5.2:*** *Schematic of the wetland experimental setup. The dashed line indicates the height of gravel, sand and sods with plants.The waterlevel was maintained just below this height. In- and effluent flowrates were controlled by peristaltic pumps. Plants are omitted from the scheme for clarity. The effluent of the wetland is subsequently treated in a BES or discharged.*

### 2.2 Bioelectrochemical system construction and operation

**The bioelectrochemical system used for producing current from wetland effluent** consisted of two Perspex frames with an inner diameter of 5 * 20 * 2 cm and a wall thickness of 2 cm sandwiched between two Perspex endplates (13 * 28 * 2 cm). The two compartments were separated by a cation exchange membrane (Ultrex CMI-7000, Membranes international Inc, USA). Rubber gaskets (3 mm thick) were used to create a watertight seal between all Perspex parts (Figure 5.3). The cathode was a custom made gas diffusion electrode (GDE) (Pant et al., 2011; Zhang et al., 2011) with an integrated current collector and a total projected area of 100 $cm^2$. The anode consisted of carbon felt (3.28 mm thick, Alfa Aeasar, Germany) and was used as received, projected area of 100 $cm^2$. The anode current collector was a steel mesh (inox AISI 304, mesh width: 5,45 mm, wire thickness: 0.8 mm, Omnimesh, Belgium) with two leads protruding through the rubber gaskets for external connections. Both anode

and cathode were placed against the membrane in order to limit diffusion resistances. Liquid connections were provided via the endplates. A Ag/AgCl reference electrode (RE-1B, Biologic, France) was inserted through the anode endplate and placed close to the anode electrode. The cathode compartment was open to the air and did not contain any liquid. Before inserting the cathode into the reactor, the membrane side was wetted to ensure adequate liquid contact. Cloth filtered (Liplisse 3 Cloth, Libeltex, Belgium) wastewater (for start-up purposes) or wetland effluent were added to the anode directly without any other treatment. Anode inoculum was the effluent of a MFC that was continuously operated in the lab for the specific purpose of providing inoculum. Cell potential over a 500 ohm resistor and anode or cathode potential were measured continuously (HP 34970A, Agilent, The Netherlands) during start-up of the BES. During experimental periods, the anode was controlled at a potential of 0 vs SHE with a potentiostat (VSP, Biologic, France). Polarization curves were recorded at a scanrate of 1 mV $s^{-1}$ following a 20 min stabilization period in open circuit. Electrochemical calculations were performed according to Logan et al. (2006).



**BES for hydrogen peroxide production.** This reactor was of the same design as the one used for current production (Figure 5.3). The anode electrode was a dimensionally stable (DSA) Ir coated Ti mesh (Ta/Ir 0.35/0.65; dimensions: 5*20*0.1 cm; specific surface area: 1 $m^2$ $m^{-2}$, Magneto Special Anodes, The Netherlands) with an integrated 5 mm diameter rod of similar material as a current collector. The cathode electrode consisted of a carbon felt (dimensions: 5*20*0.3 cm, Alfa Aesar, Germany) interwoven with 2 carbon rods (dimensions: 0.5*30 cm; P48677-CMG, Morgan, Belgium) from the short side of the carbon felt with 1.5 cm spacing. To ensure adequate electrical contact between the rods and the felt, conductive carbon cement (Leit C, Laborimpex, Belgium) was used. An anion exchange membrane (AMI-7001, Membranes international Inc, USA) was used to separate the two compartments to prevent any diffusion of metal ions towards the cathode. The Ag/AgCl reference electrode was inserted through the cathode endplate and placed close to the cathode electrode. At the bottom of the cathode compartment two inlets were foreseen to maintain the dissolved $O_2$ concentration at 8 mg $L^{-1}$. This lead to a cathode potential of ~ -0.23 V vs. SHE throughout all experiments. Anode and cathode were operated in batch with a total liquid volume of 0.5 L each and a recirculation rate of 1.08 L $h^{-1}$. The anolyte consisted of wetland effluent and two different catholytes (0.3 % NaCl and wetland effluent) were used. The electrochemical cell was operated in a galvanostatic mode (VSP, Biologic, France) for 24 h per current density and electrolyte combination. Per electrolyte combination, four current densities were tested, starting from a biological relevant current density of 2.5 A $m^{-2}$ in incremental steps of 2.5 A $m^{-2}$ up to 10 A $m^{-2}$. Between each current density/electrolyte combination the anode and cathode compartment were rinsed for at least 24 h with 0.3% NaCl.

The reference electrodes were regularly monitored versus a calomel electrode (+244 mV vs. Standard Hydrogen Electrode (SHE); QIS, the Netherlands). Removal rates, current and power densities are expressed per $m^2$ membrane projected surface area.

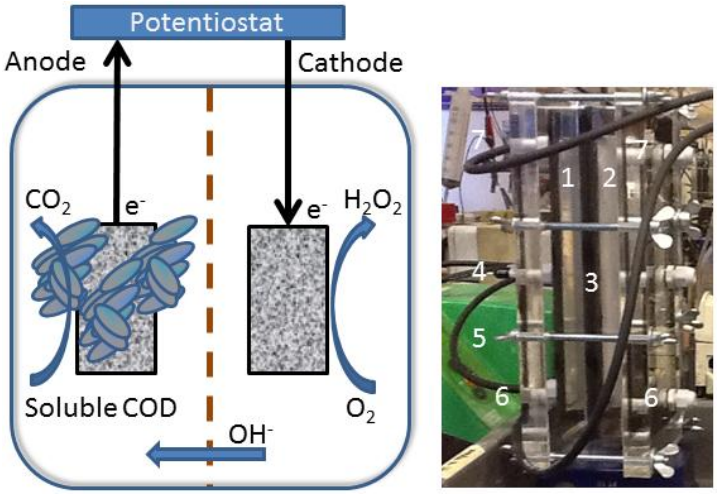



**Figure 5.3:** *General scheme (left) and photo (right) of the BES used in this work. 1) anode compartment, 2) cathode compartment, 3) rubber gaskets and membrane, 4) reference electrode and 5) peristaltic pump, 6) influent connections and 7) effluent connections.*



## 2.4 Chemical analysis

Chemical oxygen demand was determined by means of a standard kit according to the manufacturer's procedure (Nanocolor ® COD, Macherey-Nagel, Germany). Soluble COD was determined after filtration over a 0.45 µm filter. pH was determined using a handheld probe (SP10B, Consort, Belgium). Dissolved oxygen was determined with a handheld $O_2$ probe (HQ30D, Hach Lange, Germany). Hydrogen peroxide concentrations were determined by means of a spectrophotometric method adapted from O'Sullivan and Tyree (2007). Briefly, 1 ml of appropriate diluted sample (in 0.3 % NaCl) was added to 1 ml 1.8 M $H_2SO_4$ and 24 mM $TiOSO_4$*x$H_2O$ (5% Ti basis, Sigma Aldrich, Germany). Absorbance was read after 10 min. incubation at room temperature at 405 nm. A linear standard curve from 0-70 mg $L^{-1}$ was used to quantify $H_2O_2$.

## 2.5 Disinfection tests

$H_2O_2$ was directly added from the bottle to stirred wetland effluent at room temperature to final concentrations of 0.1 and 0.01%. Disinfection effectiveness was determined by (selective) plate counting and by means of flowcytometry. Samples were taken before addition of $H_2O_2$ and at 5 or 10 min. intervals up to an hour after addition of $H_2O_2$. Peroxidase (~ 3U $ml^{-1}$ final concentration) was added to the sample to stop the action of $H_2O_2$.

Appropriate dilutions were made in 8.5 g NaCl $L^{-1}$ sterile physiological solution. Total plate counts were determined on R2A agar after 24 h of incubation due to the presence of spreader colonies. Enterococci were determined after incubation for 48 h at 37°C on *Enterococcus* agar (Difco, BD, Belgium). Total coliforms were determined after overnight incubation at 37°C on MacConkey agar (Oxoid, UK). Total bacteria viability analysis based on membrane integrity was performed by means of flowcytometry according to Van Nevel (Van Nevel et al., 2013). The procedure was adjusted to 0.4 µM Propidium Iodide (PI) and 13 min. incubation at 37 °C. Presence of peroxidase did not affect flowcytometric determinations (not shown).

# 3. Results & Discussion

This chapter describes a concept with an initial filtering of wastewater via a high rate wetland, followed by the bioelectrochemical production of peroxide and subsequent disinfection of wetland effluent. To demonstrate the concept, 2 lab-scale wetlands were operated to study organics removal, secondly a BES was coupled to a wetland to study current production from wetland effluent, thirdly electrochemical $H_2O_2$ production in real wetland effluent (Aquafin, Belgium) was investigated and finally disinfection experiments with $H_2O_2$ were performed with real wetland effluent to determine the needed concentration.

## 3.1 Wetland COD removal and anode performance



The wetlands in this study produced an effluent flowrate of 21.4 ± 7.2 L $m^{-2}_{wetland}$ $d^{-1}$ at a concentration of 161 ± 53 mg $COD_{soluble}$ $L^{-1}$ (Table 5.1, stage 2). The wetland effectively operated as a filter, since suspended COD (i.e. total COD – soluble COD) removal efficiency was 95 ± 7.4 % whereas soluble COD was less efficiently removed, 72. ±6.6 %, before coupling of the BES to the effluent. In this particular case, remaining soluble COD serves as a source of reducing equivalents to drive current generation in the anode of a BES.

Comparing the loading rate of the labscale wetlands with full scale wetlands shows that the wetlands in this study received a 1-10 times higher COD loading rate at the later stages (Kern & Idler, 1999; Karathanasis et al., 2003; Puigagut et al., 2007). This indicates that loading rates can be increased on existing wetlands when aiming only at quick filtration. An important issue that still needs to be addressed is the prevention of clogging of the gravel bed in the long run. This can possibly be achieved by using a larger gravel size or adjusted the loading rate. Moreover, full scale wetlands are usually operated with a pretreatment step (Kern & Idler, 1999; Karathanasis et al., 2003; Puigagut et al., 2007). COD effluent concentrations of the labscale wetland were in compliance with effluent concentrations according to Belgium regulations (< 125 mg$COD_{total}$ $L^{-1}$) however relative removal needs to be improved (total COD removal efficiency of 70% is needed) (Rousseau et al., 2004; Vlarem_II, 2012). Additional COD removal was achieved by the anode of the BES (Table 5.1). This resulted in an average biologically generated current of 58 mA $m^{-2}$ (Figure 5.4, till day 25) from the effluent of the wetland and an extra 36% decrease of effluent $COD_{total}$ concentrations (Table 5.1, stage 2 vs. stage 3).

Coulombic efficiency during this period amounted to 4.0 % indicating that also other processes played a role, such as settling of solids or conversion with other electron acceptors. Bioanode performance was limited by the amount of $COD_{soluble}$ available, as spiking (starting day 25) of the wetland influent yielded higher current densities, up to maximum 3.5 A $m^{-2}$, equal to 25 gCOD $m^{-2}$ $d^{-1}$ with coulombic efficiency of 62% (Figure 5.4a, Table 5.1; Stage 4). This indicates that the wetland/ BES combination was underloaded in terms of anode performance. This finding is corroborated by polarization curves (Figure 5.4b) where the anode potential changed more at higher currents compared to the cathode potential (2.7 times more change in anode potential vs. cathode potential at currents > 0.95 A $m^{-2}$). The higher loading of the wetland/BES combination ( ~ 50%, Table 5.1 stage 3 and 4) resulted in a net power output during polarization of maximum 150 mW $m^{-2}$ (Figure 5.4c).

The higher current densities achieved during this work, as compared to the previous chapters, show the advantage of concentrating the electron donors in a relatively small anode compartment. Here,

the anode was part of a reactor system, where fluid flow and mixing were able to provide higher concentrations of organic matter to the microorganisms and remove excess protons from the anode surface. This is in contrast to the sediments systems where fluid flow and mixing is rather limited. Moreover, the reactor system in this chapter was operated under potentiostatic control, removing any influence of the cathode possibly negatively impacting the anode reactions.

The higher $COD_{soluble}$ content in the influent led to an increase of the $COD_{soluble}$ concentration in the effluent of the BES, surpassing the discharge limit (Rousseau et al., 2004; Vlarem_II, 2012). In the proposed concept (Figure 5.1) a second wetland will provide a polishing step to remove residual organics and nutrients so discharge limits can be met.



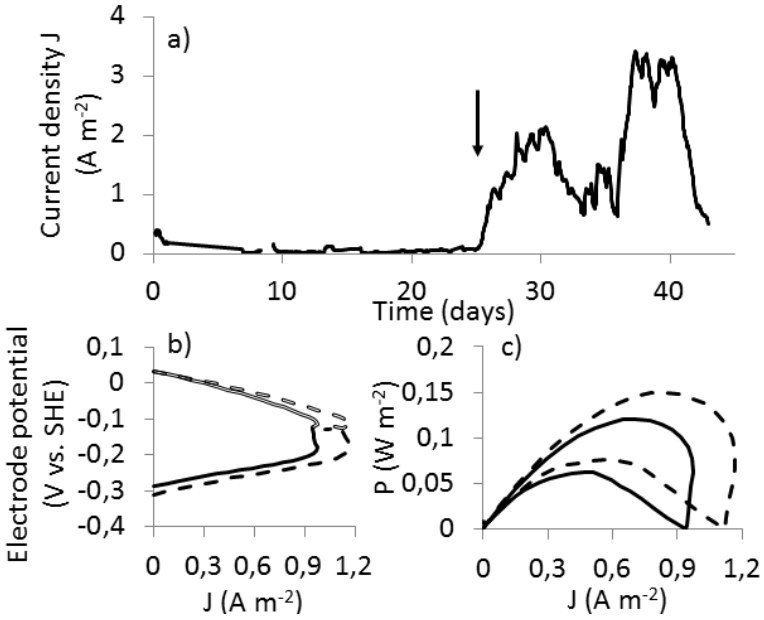



***Figure 5.4:*** *a) Current density in function of time for a BES operated on wetland effluent. Arrow indicates start of spiking wetland influent, see § 3.1. b) anode (solid lines) and cathode (open lines) potentials and c) power density curves during polarization measurements on day 36 (– – .) and 42 (——).*

## 3.2 Peroxide production in wetland effluent.

From an applied perspective, producing peroxide directly in wetland effluent is the most advantageous option. This does not require a separate cathodic liquid supply and not an extra unit operation to provide sufficient mixing and contact time for the separately produced peroxide and the wetland effluent. For this reason peroxide production in wetland effluent was compared to production in 0.3 % NaCl at various current densities. 0.3 % NaCl was chosen to compare with other studies on bioelectrochemical peroxide production(Rozendal et al., 2009; Modin & Fukushi, 2013). A maximum rate of peroxide production, 2.7 g $m^{-2}h^{-1}$, was achieved at 10 A $m^{-2}$ after 4 hours in the batch cycle. No clear difference was observed between a catholyte of 0.3% NaCl or wetland effluent (Figure 5.5a). This indicates that there is little need for an additional cathodic water supply, thus disinfectant can be produced *in situ*. The maximum hydrogen peroxide production rate was achieved at a cathodic coulombic efficiency of 40%. Lower rates had higher cathodic coulombic efficiencies

with a maximum efficiency of 51% achieved at 1.7 g $m^{-2}$ $h^{-1}$ at a current density of 5 A $m^{-2}$ after 7 hours contact time (Figure 5.5b). In all cases a contact time of 24 h was too long in terms of overall rate and efficiency (Figure 5.5b). Other works have shown that peroxide production rates can be increased by ~ 33% (Modin & Fukushi, 2013) and efficiencies by ~ 40% (Figure 5.5). These improvements can be mainly attributed to reactor design (5 ml cathode vs. 500 ml, this work) and the use of cathode material (gas diffusion electrodes (Rozendal et al., 2009; Modin & Fukushi, 2013) vs. off the shelf carbon felt, this work). The combination of the anion exchange membrane with the high cathodic pH caused a minor transfer of hydroperoxyl to the cathode compartment with a maximum anodic peroxide concentration of 0.0015% after 24 h at 10 $Am^{-2}$, some 2.6% of the total peroxide quantity produced.

***Table 5.1:*** *Overview of COD and flow rates on both wetlands and the coupled system.*

| Stage | 1) start-up | | | 2) increase solids | | | 3) coupling of BES to effluent | | | | | 4) increase soluble organics | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wastewater source | Domestic wastewater | | | Hospital wastewater | | | Hospital wastewater | | | | | Hospital wastewater | | | | |
| Operating days | 36 | | | 47 | | | 25 | | | | | 18 | | | | |
| | **WL1** | **WL2** | **n** | **WL1** | **WL2** | **n** | **WL + BES** | **n** | **WL** | **n** | **BES** | **n** | **WL + BES** | **n** | **WL** | **n** | **BES** | **n** |
| *Loading rate (gCOD $m^{-2} d^{-1}$)* [†] | | | | | | | | | | | | | | | | | | |
| total | 20.3 ± 2.6 | 20.2 ± 2.3 | 4 | 26.6 ± 15.5 | 29.9 ± 17.7 | 9 | 19.1 ± 9.1 | 5 | | | | | 44.2 ± 23.5 | 5 | | | | |
| solids | 2.3 ± 1.1 | 1.7 ± 1.6 | 4 | 12.6 ± 8.0 | 15.9 ± 10.6 | 9 | 9.2 ± 9.4 | 4 | | | | | 5.5 ± 4.5 | 4 | | | | |
| soluble | 18.0 ± 2.9 | 19.0 ± 3.6 | 4 | 14.0 ± 9.0 | 13.9 ± 8.5 | 9 | 12.6 ± 5.3 | 6 | | | | | 46.5 ± 21.3 | 4 | | | | |
| *Removal rate (gCOD $m^{-2} d^{-1}$)* [†] | | | | | | | | | | | *(gCOD $m^{-2} d^{-1}$)** | | | | | | *(gCOD $m^{-2} d^{-1}$)** | |
| total | 15.6 ± 1.7 | 15.7 ± 1.8 | 3 | 23.5 ± 14.0 | 26.7 ± 18.2 | 7 | | | 18.1 ± 15.4 | 2 | 10.6 ± 6.3 | 2 | | | 30.8 ± 21.9 | 5 | 40.5 ± 44.5 | 5 |
| solids | 2.1 ± 1.3 | 2.1 ± 0.2 | 3 | 12.4 ± 7.9 | 16.7 ± 11.9 | 7 | | | 12.6 ± 14.3 | 2 | 5.1 ± 5.8 | 2 | | | 6.1 ± 4.0 | 2 | 20.3 ± 39.2 | 4 |
| soluble | 13.5 ± 0.4 | 13.6 ± 1.7 | 3 | 11.1 ± 7.6 | 12.9 ± 8.2 | 7 | | | 5.6 ± 1.2 | 2 | 5.7 ± 0.5 | 2 | | | 32.5 ± 26.2 | 4 | 44.7 ± 34 | 5 |
| *Flow rate (L $d^{-1}$)* | | | | | | | | | | | | | | | | | | |
| in | 10.6 ± 1.3 | 10.6 ± 1.4 | 6 | 11.2 ± 5.6 | 11.1 ± 5.4 | 16 | | | 14.3 ± 3.4 | 10 | 4.3 ± 1.0 | 11 | | | 10.8 ± 5.5 | 8 | 4.8 ± 2.9 | 8 |
| out | 5.5 ± 2.4 | 6.3 ± 3.1 | 9 | 5.8 ± 2.0 | 5.5 ± 2.9 | 20 | | | 10.6 ± 2.0 | 8 | # | | | | 9.3 ± 2.4 | 8 | # | |
| *Effluent concentration (mgCOD $L^{-1}$)* | | | | | | | | | | | | | | | | | | |
| total | 81.0 ± 58.4 | 130.0 ± 45.5 | 12 | 187.2 ± 89.8 | 164.7 ± 152.9 | 12 | | | | | 98.0 ± 32.2 | 5 | | | | | 267.4 ± 75.3 | 5 |
| solids | 6.1 ± 6.5 | 21.4 ± 26.5 | 12 | 28.2 ± 51.4 | 16.9 ± 34.7 | 8 | | | | | 11.8 ± 5.7 | 4 | | | | | 9.6 ± 8.1 | 5 |
| soluble | 81.5 ± 47.8 | 109.2 ± 50.2 | 12 | 160.5 ± 53.3 | 160.4 ± 186.4 | 13 | | | | | 93.2 ± 31.2 | 6 | | | | | 257.8 ± 71.7 | 5 |

[#]: in = out in the BES.

*: rates for the BES are calculated per projected membrane surface area.

[†]: loading rate considered for a bed height of 30 cm.

WL: Wetland

BES: Bioelectrochemical system

n: number of samples

Instead of using a biologically generated current, one can opt for a pure electrochemical system. In the case of the highest achieved rate at 10 $Am^{-2}$ an energy investment of 6 W $m^{-2}$ was needed. This amounts to 2.5 kWh $kg_{peroxide}^{-1}$ and with an assumed energy price of € 0.05 $kWh^{-1}$, a minimum production cost of € 0.11 $kg_{peroxide}^{-1}$. Other benefits of electrochemical peroxide production include the possibility of generating active chlorine compounds at the anode electrode but care should be taken to limit the occurrence of disinfection by-products (Bagastyo et al., 2011). When using wetland effluent as the water source for the anode and cathode, which has a low buffer capacity, an increase in cathodic pH and decrease in anodic pH was readily observed (Figure 5.3c). This phenomenon is usually regarded as a drawback for use of BES, in other applications due to associated energy losses (Rozendal et al., 2006a), but here it can become a positive attribute as it will also aid in disinfection.

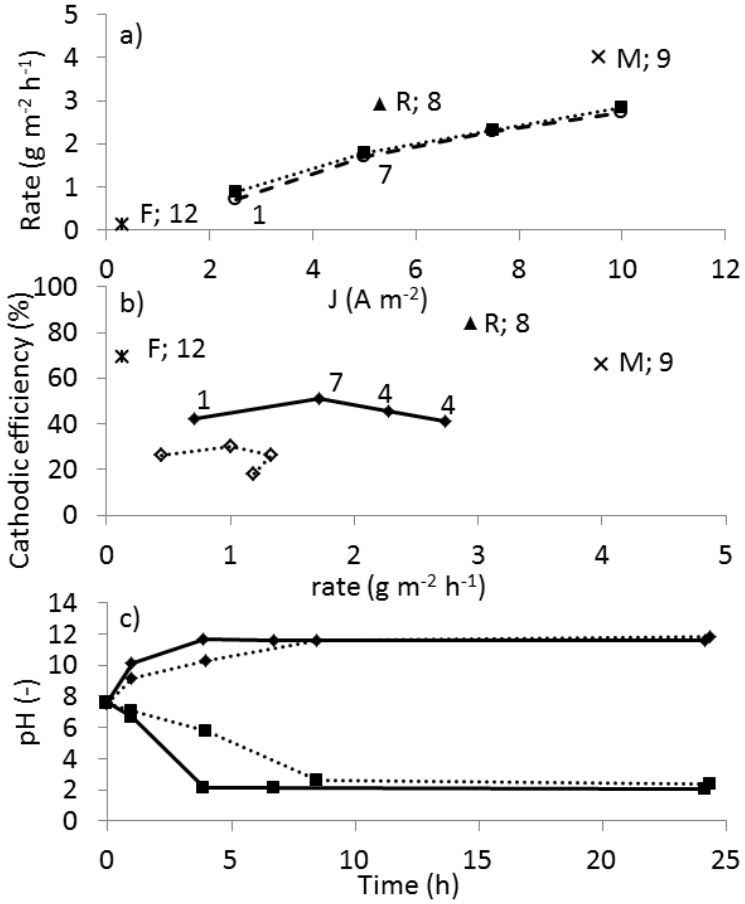



***Figure 5.5:*** *a) hydrogen peroxide production rates at increasing current densities with 0.3% NaCl (··■··) and wetland effluent as catholyte (–⊖·). All data points are maximum rates obtained at 4 hours contact time except where numbers are present next to data points. b) Maximum (–◆–) and 24h (···◇···) rate of hydrogen peroxide production in function of cathodic coulombic efficiency. Time of maximum rate is indicated. c) pH profile for the anode (■) and cathode(◆) compartment during $H_2O_2$ production in wetland effluent at 2.5 $Am^{-2}$ (.....) and 10 $Am^{-2}$(——). Outcomes of comparable studies: F: Fu et al. (2010). R: Rozendal et al. (2009). M: Modin et al. (2013).*

### 3.3 Peroxide requirement for treatment of wetland effluent

The concentration of hydrogen peroxide needed for effective disinfection determines the required quantity and thus the cathodic production rate and further BES dimensions. 0.01% hydrogen peroxide lead to a 50% removal of total bacterial counts within wetland effluent on R2A agar after 1 hour contact time. Increasing the concentration 10 times to 0.1% lead to an almost complete removal of culturable bacteria (3 log reduction to <75 CFU $ml^{-1}$) after 1 h contact time (Figure 5.6a.). These results are in line with pure culture kinetic and modelling studies (Labas et al., 2008; Vargas et al., 2013). A similar trend is observed for total coliforms as determined by selective plating on MacConkey agar (Figure 4a). No culturable *Enterococci* were detected by means of selective plating on enterococcus agar (<75 CFU $ml^{-1}$).

Flowcytometric analysis with viability staining indicated a far less efficient removal as compared to selective plate counts. Both concentrations of disinfectants showed a similar increase in damaged cell counts (Figure 5.6b). Examining the increase in percentage of dead cells over time reveals a similar pattern with little difference between both concentrations (Figure 5.6c) with a maximum increase in dead cells of 35% after 40 min contact time for 0.1% $H_2O_2$. The difference between plate counts and flowcytometric results indicates that although cell membrane integrity seems to be intact, the microorganisms are damaged sufficiently to not be viable and/or culturable on agar plates (Hoefel et al., 2003).



### 3.4 Wetland & BES dimensions and configuration

With the results presented here a case study can be made on the design of a constructed wetland for water treatment. Considering a municipality of 750 person equivalents (PE), producing 100 L with 0.5 gCOD $L^{-1}$ wastewater per inhabitant (Kern & Idler, 1999; Vymazal, 2005) which results in a flowrate of 75 $m^3$ $d^{-1}$ or a mass flowrate of 37.5 kgCOD $d^{-1}$. At the highest loading rate as determined in this work (45 gCOD $m^{-2}$ $d^{-1}$, Table 5.1), 850 $m^2$ of wetland is needed to filter the solids from the wastewater flow. This will lead to a wetland effluent of 170 mgCOD $L^{-1}$. The COD in the effluent can be further treated with an anode. Taking a BES and operating it with a bioanode producing 2.5 $Am^{-2}$ at 40 % coulombic efficiency (suboptimal conditions from the maxima reported earlier) leads to the notion that some 44 gCOD $m^{-2}$ $d^{-1}$ can be processed. When using a membrane electrode assembly (MEA) of 1*0.05*1 $m^3$ (5 cm width for one assembly is a reasonable estimate (Dekker et al., 2009; Cusick et al., 2011)), 880 gCOD can be treated per $m^3$ per day. To treat the complete COD load of the wetland effluent, a 15 $m^3$ reactor would be needed. The cathode was able to produce 17 $gH_2O_2$ $m^{-2}$ $d^{-1}$ at 2.5 $Am^{-2}$ (Figure 5.5a ), one $m^3$ of BES will thus produce 340 $gH_2O_2$ $d^{-1}$. To achieve a good disinfection (i.e. viable heterotrophic count < 100 CFU $mL^{-1}$ (98/83/Ec, 1998)), 0.1% $H_2O_2$ was needed in this work (Figure 5.6a). However other wastewaters might require different concentrations of disinfectant, depending on their initial constituents. With the water tested in this work 340 L $d^{-1}$ of disinfected water can be produced per $m^3$ of reactor (or 5.1 $m^3$ for the 15 $m^3$), if the purpose is to produce water for consumption. If the purpose is to lower the infectious pressure e.g. in a horticulture scenario with irrigation water recycling, depending on the needed concentration of $H_2O_2$, a higher flow of water can be produced. Moreover, setting the current density irrespective of anode performance, can lead to higher rates of $H_2O_2$ production (Figure 5.5a). With the data provided by Rozendal et al. (2008b), the total installed reactor costs can be estimated at € 5800,- for a system with 20 MEA in a $m^3$.

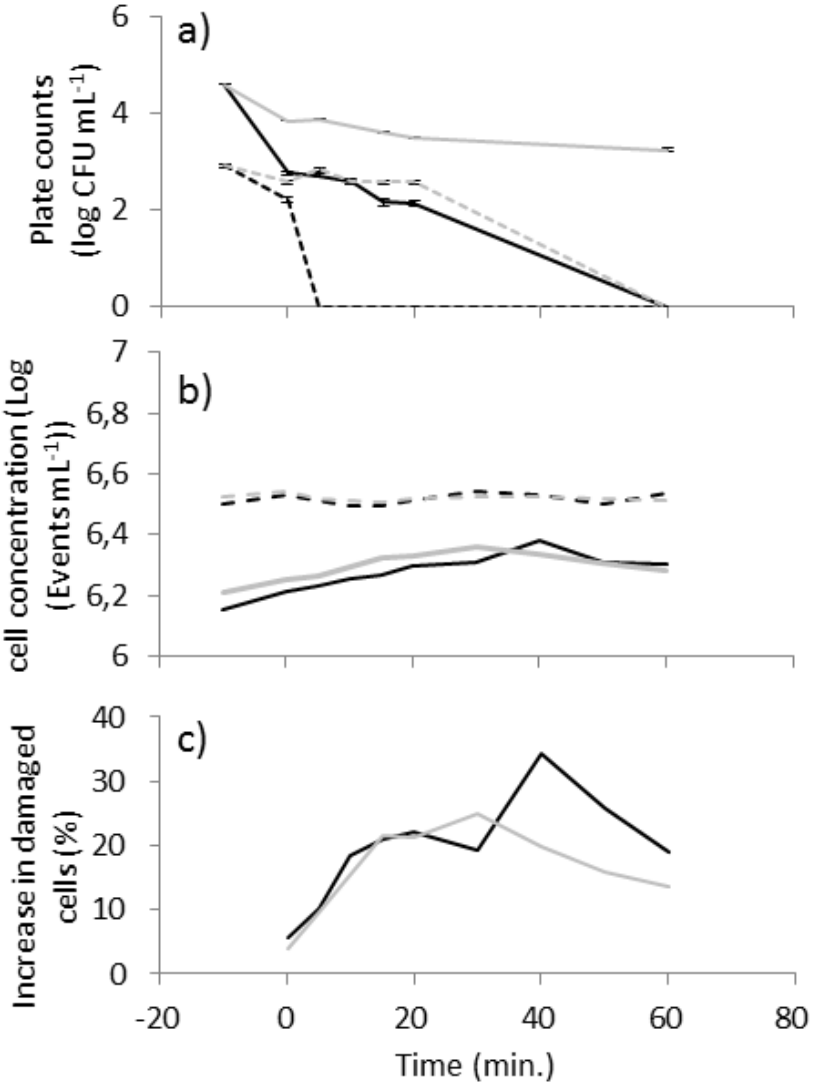



***Figure 5.6:*** *Disinfection performance of 0.1% and 0.01% $H_2O_2$ on wetland effluent. a) plate counts 0.1% total bacteria: ——, 0.1% coliforms: - - - -, 0.01% total bacteria: ——, 0.01% coliforms: - - - -. Error bars indicate 95% confidence interval as determined with the Poisson distribution, not all error bars are visible. Values at 0 are below detection limit of 75 CFU $ml^{-1}$ for total bacteria and 150 CFU $ml^{-1}$ for coliforms. b) Flow cytometry based viability staining for 0.1% and 0.01% $H_2O_2$. Intact cells 0.1%: - - - - 0.01%: - - - -, damaged cells 0.1%: —— 0.01%: ——, c) increase in dead cells for 0.1% and 0.01% $H_2O_2$.*

## 4. Conclusions

In this work a process train for wetland wastewater treatment with subsequent disinfection via (bio)electrochemical $H_2O_2$ production was studied. The lab scale wetland was able to operate with loading rates up to 45 gCOD $m^{-2}$ $d^{-1}$ and provide an almost solid free effluent to the anode of a bioelectrochemical system (BES). The anode of the BES was in short supply of organic carbon showing opportunities to increase the load on the wetland. Production of $H_2O_2$ for disinfection was shown to be feasible directly in wetland effluent up to rates of 2.7 g $m^{-2}$ $h^{-1}$ with additional benefits of pH gradients in the BES. Wetland effluent could be disinfected with 0.1% $H_2O_2$ and a contact time

of 1 h. Finally, a scalable integrated system configuration is proposed that can be expanded according to the need for clean water.

## Acknowledgements

JA was supported by the European Community Seventh Framework Programme FP7/2007-2013 under grant agreement no.226532. The authors thank Karen de Roy, Jo De Vrieze and Sam van Nevel for helpful suggestions and fruitful discussions. Deepak Pant (VITO, Belgium) is kindly acknowledged for providing of the gas diffusion electrodes. Nancy Marchant (Aquafin, Belgium) is kindly thanked for access to the various wastewater treatment plants. Andre van de Velde is thanked for access to the Maria Middelares wastewater treatment plant (Gent, Belgium).

# CHAPTER 6: MICROBIAL ELECTROSYNTHESIS OF ACETATE FROM $CO_2$ IS POSSIBLE ON BOTH CARBON AND STEEL CATHODES

This chapter has been redrafted after:

Arends, J.B.A., Boon, N., Rabaey, K. 2013. Microbial electrosynthesis of acetate on carbon or steel cathodes: Is there a difference? *Submitted*

## Abstract

Microbial electrosynthesis is an electricity driven process to produce organic compounds from $CO_2$ and precursor organics using microorganisms as catalysts. The key example is acetate production from $CO_2$, typically at a carbon cathode using immobilized bacteria on the cathode. However, a key question remaining is whether this attachment of cells is necessary to achieve effective bioproduction. Stainless steel and carbon cathodes were compared at a fixed current density using *Acetobacterium woodii* as a biocatalyst. Acetate production rates of 536 ± 226 mg $m^{-2}$ $h^{-1}$ for a steel cathode and 340 ± 131 mg $m^{-2}$ $h^{-1}$ for a carbon cathode were achieved. Operation at a fixed current density greatly increased production rates per unit projected surface at similar energy investment per gram of product as compared to previously reported studies at set potentials. Whereas further improvements are needed to make this process competitive with existing approaches, these results show that *in situ* hydrogen production with steel has the potential to rapidly transfer electrons to planktonic cells.

## 1. Introduction

In the search for renewable fuels and sustainable bioproduction processes, a new technology platform termed microbial electrosynthesis (MES), based on bioelectrochemical systems (BES), has been proposed (Nevin et al., 2010). The concept relies on the use of an electrical current to or from a solid electrode to a microbial catalyst to drive a bioproduction process. The narrow definition of MES spans only *de novo* synthesis with $CO_2$ as a feed source (Nevin et al., 2010). The broad definition also includes reducing feed components like glycerol (Dennis et al., 2013) or driving an unfavourable oxidation by removing excess reducing equivalents (Flynn et al., 2010). The use of $CO_2$ for bioproduction by means of MES has multiple advantages (o.a. land independent production, abundant supply) and disadvantages (o.a. low concentration in atmosphere, $CO_2$ is fully oxidized) as detailed elsewhere (Desloover et al., 2012). The principles, technical considerations and possible applications have already been widely reviewed (Rabaey & Rozendal, 2010; Lovley, 2011b; Rabaey et al., 2011; Desloover et al., 2012; Marshall et al., 2013b) but few experimental reports have been published thus far.

The main focus of experimental research has till now been the reduction of $CO_2$ to acetic acid. Several axenic homoacetogenic cultures (Nevin et al., 2010; Nevin et al., 2011b) and mixed communities (Marshall et al., 2012; Su et al., 2013) were able to accept electrons from an electrode and produced mainly acetate by means of reducing $CO_2$. Carbon based electrodes (known for higher overpotentials compared to stainless steel for hydrogen evolution (Call et al., 2009)) were used in all studies due to their perceived biocompatibility and better facilitation of cell adherence (Nevin et al., 2010; Nevin et al., 2011b; Marshall et al., 2012; Zhang et al., 2013). In other contexts stainless steel cathodes have been used to e.g. drive biological oxygen reduction reactions in marine environments (Dumas et al., 2008a). Stainless steel cathodes have been used for MES processes to facilitate the reduction of fumarate to succinate (Dumas et al., 2008b) and the production of glycerol from succinate by a *Geobacter sulfurreducens* (Soussan et al., 2013). The described experiments aimed at direct electron transfer by setting the cathode potentials to values assumed to be unfavourable for the hydrogen evolution reaction (HER). It still remains unclear what the impact of hydrogen uptake by microorganisms close to the electrode would be on the necessary potential. Direct electron transfer is an interesting feature from a fundamental point of view. Next to the fundamental considerations, from a practical perspective direct electron transfer is convenient since high coulombic efficiencies can be obtained (no assumed hydrogen loss) and the biocatalyst is retained on the electrode. Considering that most studies applied a cathodic potential of -0.4 V vs. the standard hydrogen electrode (SHE) and relied on $O_2$ evolution at the anode ($E'_0$ = 0.82 V vs. SHE) it can be calculated that at 20 A $m^{-2}$ (Soussan et al., 2013) a power input of 24.4 $Wm^{-2}$ projected cathode surface is needed. When aiming for hydrogen evolution at least an additional 0.2 V is needed (Aulenta et al., 2008), leading to a power input of 28.4 W $m^{-2}$. An increase in power input of 16% thus leads to the possibility of creating two products i.e. organic products (type depending on the biocatalysts) and a gaseous fuel ($H_2$) as a "loss". Moreover, it would enable the use of planktonic cells (using the $H_2$) for the production process which is more in line with conventional bioproduction approaches. In this work the possibility of using a stainless steel felt cathode was evaluated relative to a graphite felt cathode. *Acetobacterium woodii* was used as the model organism as it is unable to directly accept electrons from the cathode, possibly due to its sodium based ATPases (Nevin et al., 2011b) and was identified as a dominant organism in mixed community studies (Marshall et al.,

2013a; Su et al., 2013). With this comparison the "carbon electrodes for attached biocatalysts" paradigm is challenged.

## 2. Materials and Methods

### 2.1 BES construction and operation

Five-necked glass electrochemical reactors with 150 ml liquid volume were used for all experiments. The middle neck housed a glass tube with a 0.79 $cm^2$ membrane (Ultrex CMI-7000, Membranes International Inc., USA.). The inside acted as the anode compartment with a dimensionally stable anode consisting of $TaO_2/IrO_2$ (35/65%) coated Ti mesh anode (dimensions: 5*2*0.1 cm; specific surface area: 1 $m^2$ $m^{-2}$, Magneto Special Anodes, The Netherlands) for water electrolysis. The other four necks were used to connect gas ($N_2/CO_2$; 90%/10%) in- and effluent ports, a liquid sample port, a capillary filled with (3 M KCl) to hold a reference electrode (RE-1B, Biologic, France) and the working electrode (cathode). The cathode consisted of either a stainless steel felt (316L web ~8 µm fibers, 2.5 * 2 * 0.3 cm, Bekaert, Belgium) connected to a stainless steel wire current collector or a carbon felt (2.5 * 2 * 0.33 cm, Alfa Aesar, Germany) connected to a carbon rod (dimensions: 0.5*30 cm; P48677-CMG, Morgan, Belgium). To ensure adequate electrical contact between the rod and the felt, conductive carbon cement (Leit C, Laborimpex, Belgium) was applied. Sterile gas was supplied thorough filters in the gas lines (1 µm pore size, Millipore, USA). A constant current of -0.15 mA $cm^{-2}$ was applied using a potentiostat (VSP, Biologic, France). Potentials are reported versus the Ag/AgCl reference electrode (3 M KCl). Cyclic voltammograms were recorded at a scan rate of 1 $mVs^{-1}$.



### 2.3 Media and microorganism cultivation

*Acetobacterium woodii* (DSM1030) was routinely cultivated in standard medium (DSM medium 135) at 28 °C under static conditions. During experiments in the BES, all organic carbon, resazurin and reducing agents were omitted. Adjusting the pH 7.9 ±0.2 was done with 1 M HCl in cultivation medium when needed. Upon sampling, the same volume of medium salts, omitting trace elements and vitamins was added. The anolyte consisted of 5 mM $Na_2SO_4$ adjusted to pH 2 with $H_2SO_4$. The BES was operated at room temperature (26 ± 2 °C) under mild mixing conditions with a stirring plate. Each reactor was polarized for at least 1 day before inoculation to ascertain sterility and anaerobic conditions

### 2.4 Chemical analysis

Volatile fatty acids were analyzed using an AS1 column in a DX-500 BioLC with an ED50 conductivity detector (Dionex). The eluent was 0.4 mM HCl in milliQ water. Off-gas was analyzed with a gas chromatograph (CompactGC, Global Analyser Solutions, The Netherlands) with a thermal conductivity detector (TCD). The bicarbonate concentration was estimated by adding 1 ml of spent cathode medium to 1 ml 1M $H_2SO_4$ in a vacutainer. Headspace $CO_2$ in the vacutainers was subsequently determined after 24 h incubation at 20 °C at 110 rpm by gas chromatography and recalculated assuming all $CO_2$ was $HCO_3^-$. Current density and production rates are expressed per $m^2$ projected cathode area (Logan et al., 2006).

Before inoculation, total cell count of the inoculum was performed via flow cytometry viability staining (Van Nevel et al., 2013). The procedure was adjusted to 4 µM Propidium Iodide (PI) and 13

min. incubation at 37 °C. Cathodes were inoculated with an axenic culture of $10^7$ live cells $mL^{-1}$. Axenity was regularly checked using phase contrast microscopy (Balch et al., 1977). Fluorescent microscopy samples were prepared by spreading a piece of felt on a glass slide, adding 20 µl dye mix i.e. Sybr Green I (SG; 200 x diluted from stock, Invitrogen ) and PI (200 µM, Invitrogen) with subsequent incubation at 37 °C for 30 minutes. Images were only adjusted on brightness/contrast levels (Image J V1.46r, USA).

## 3. Results & Discussion

The gas flow provided $CO_2$ gas resulted in a liquid concentration of 1.56 ± 0.12 and 1.96 ± 0.43 $gHCO_3^-$ $L^{-1}$ for the stainless steel and carbon cathode reactors respectively. By applying a fixed current density of -0.15 mA $cm^{-2}$, a steady flow of reducing equivalents is produced by the cathode. This resulted in the production of acetate at a steady rate of 536 ± 226 mg $m^{-2}$ $h^{-1}$ for a stainless steel cathode and 340 ± 131 mg $m^{-2}$ $h^{-1}$ for a carbon electrode (Figure 6.1), without apparent lag phase. The coulombic efficiency of acetate production was 113 ± 48 % for the stainless steel cathode and 69 ± 27 % for the carbon cathode, calculated starting day 4. Except for a limited amount of formate, a precursor of acetate production during homoacetogenesis, no other fatty acids could be detected. Formate concentrations reached maximum values of 12.8 mg $L^{-1}$ for the steel cathode (Day 7) and 50.2 mg $L^{-1}$ for the carbon cathode (Day 4) (Figure 6.1). The potentials of both electrodes were stable throughout the experiment with an hourly average cathode potential of -0.91 ± 0.02 V vs. Ag/AgCl for the stainless steel cathode and -0.91 ± 0.06 V vs. Ag/AgCl for the carbon cathode.



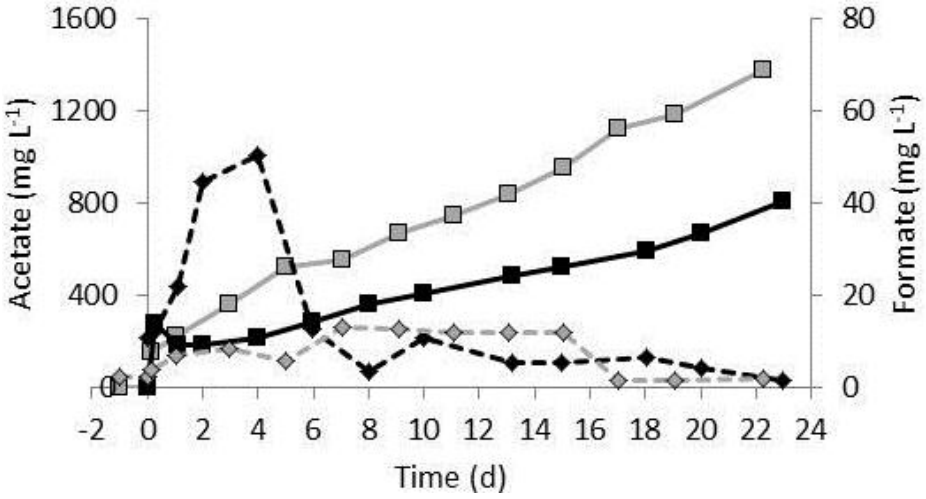



***Figure 6.1:*** *Acetate (solid lines) and formate (interrupted lines) production by A. woodii on stainless steel (grey lines) and carbon cathodes (black lines). No production was observed before inoculation (day 0). Concentrations of VFA on day 0 are due to carry over from the inoculum.*

The cyclic voltammograms recorded at the start and at the end of the experiments indicate that $H_2$ evolution was the main mechanism for electrode transfer (Figure 6.2). No catalytic events can be discerned for the potentials and current densities used during the incubation period for the stainless steel electrode. The combination of a carbon electrode with *A. woodii* enhanced the $H_2$ evolution as the onset potential of $H_2$ evolution reaction has shifted with ~ 200 mV towards the positive value. Similar findings were reported in other work that used carbon based electrodes with mixed cultures dominated by *Acetobacterium* spp. (Marshall et al., 2013a; Su et al., 2013). *A. woodii* has been

implicated not to be able to directly take up electrons from a surface (Nevin et al., 2011b), therefore the suggestion is made that *A. woodii* is able to decrease the overpotential of the HER at carbon electrodes, possibly by efficiently removing any produced $H_2$ gas from the electrode surface.

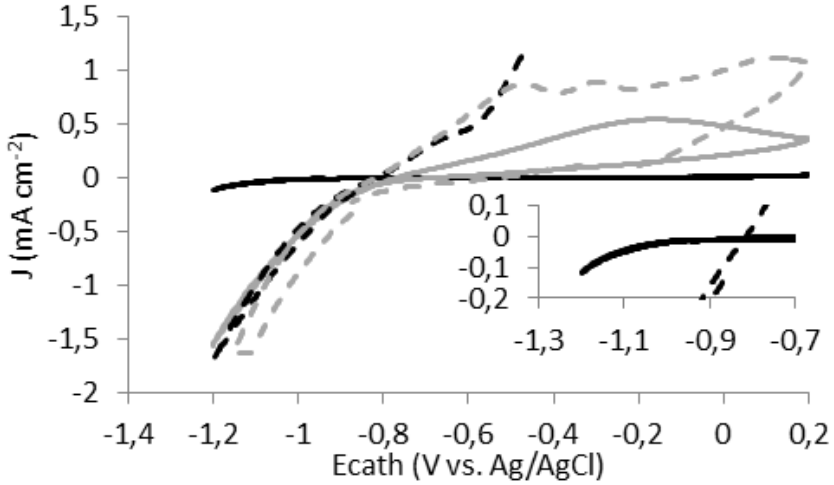



***Figure 6.2:*** *Cyclic voltammograms recorded at day 0, just after inoculation (solid lines) and at day 22 (dashed lines) of* Acetobacterium woodii *on stainless steel (grey lines) and carbon cathodes (black lines). The 2nd cycle of 4 is shown, except for carbon day 22 where the 1st cycle is shown. Inset: close-up of carbon electrode, same units apply.*

Microscopic analysis of the cathodes showed aggregates and single cells attached to the felt cathodes (Figure 6.3). This images showed that not only immobilisation of the inoculum occurred, but also attachment of single cells was possible on both carbon and steel cathodes. However, the occurrence of individual attached cells was minimal in comparison to flocks attachment (Figure 6.3). Microscopic observations, CV analysis and the use of *A. woodii* leads to the conclusion that hydrogen production was the key route for electron transfer in our system. Further research needs to elucidate whether adhered cells can, over time, further colonize the surface and change the cathode catalysis in a more direct manner.

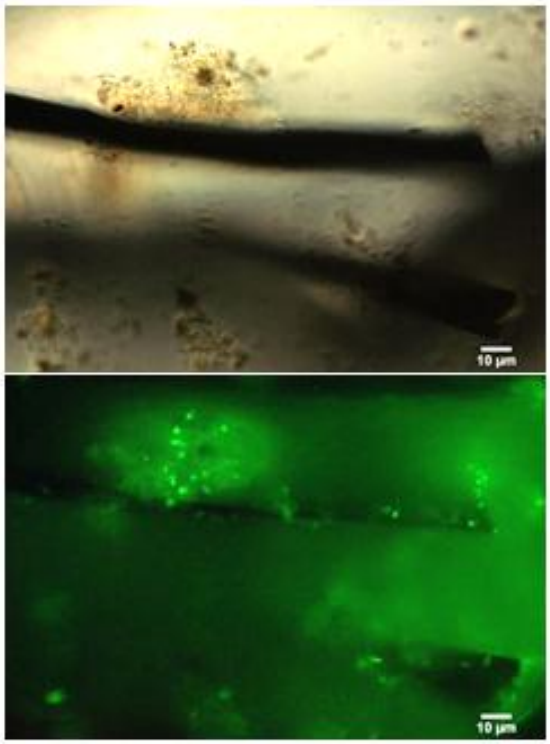



***Figure 6.3:*** *a) light microscopy image of a fibre section of a carbon cathode. b) Sybr Green I stained image of the same section.*

Comparing outputs achieved in this work with other studies on acetate production by means of MES (Table 6.1) showed that (i) energy needs are comparable with other studies (ii) higher rates can be achieved when operating at a set current density compared to set potential, indicating that electron supply is limiting and (iii) stainless steel is able to more efficiently transfer electrons to *A. woodii* as the rate of acetate production is 37% higher compared to carbon felt wen operated at a fixed current density . This leads to a necessary revisit of the paradigm that carbon cathodes and attached cells are needed for MES. Planktonic cells, not forming biofilms, maintain better electrode porosity, allowing the use of the complete reactor volume for production as opposed to the boundary layer around the electrode. Therefore bioelectrochemical systems can act as a bypass for a more conventional fermenter. Steel and other metals come at low cost, high conductivity and almost unlimited structural diversity. Thus, it can be concluded that both carbon and metal options need to be further explored.

Even in the absence of a suitable unit for comparison (e.g. surface versus volume based rates) it is clear that rates need to be tremendously improved when comparing MES to regular fermentation processes or chemical industry established processes (Table 6.1). Rates of biological acetate production can be enhanced by feeding more current, which will likely lead to higher local partial pressure of hydrogen and in turn favourable thermodynamics and kinetics. For example, a volumetric production rate of 7.4 g $L^{-1}$ $d^{-1}$ with a maximum acetate concentration of 44 g $L^{-1}$has been achieved by supplying a pure culture of *A. woodii* with 1700 mBar $H_2$ which corresponds at pH 7 to a potential of -0.57 V vs. SHE (Demler & Weuster-Botz, 2011).

## 4. Conclusions

In this work carbon and stainless steel cathodes were evaluated for use in microbial electrosynthesis by *Acetobacterium woodii*. A stainless steel cathode showed higher acetate production rates compared to a carbon cathode, most likely due to a more active $H_2$ evolution at the set current density. Operation at a set current density reveals higher production rates per surface area of electrode at similar energy expenditure per gram of product compared to operation at set potential.

## Acknowledgements

JA was supported by the European Community Seventh Framework Programme FP7/2007-2013 under grant agreement no.226532. KR and JA are supported by the European Research Council via Starter Grant ELECTROTALK. The authors thank Sunil Patil and Antonin Prevoteau and the MES-cluster for helpful suggestions and critical discussions. The contribution of Jana De Bodt in anaerobic culture work is kindly acknowledged.

***Table 6.1:*** *Overview of acetate production processes in relation to their operational parameters.*

| Reactor | | J | Ecath | Rate | | Efficiency | Electrical input | Reference |
|---|---|---|---|---|---|---|---|---|
| Biocatalyst | electrode | (mA $m^{-2}$) | (V) | (mg $m^{-2}$ $d^{-1}$) | (mg $L^{-1}$ $d^{-1}$) | (%) | (Wh $g^{-1}$ [a]) | |
| *A. woodii* | stainless steel felt | 1500 | -0.91 $^{Ag/AgCl}$ | 12865 | 46 | 128 | 4.76 | This work |
| | carbon felt | 1500 | -0.91 $^{Ag/AgCl}$ | 8156 | 29 | 81 | 7.46 | |
| Enriched community (Activated sludge) | graphite granules [b] | n.a. | -0.59 $^{SHE}$ | n.a. | 11500 [c] | 69 | 8.23 | (Marshall et al., 2013a) |
| | | n.a. | | n.a. | 6667 [d] | 50 | 11.36 | |
| Enriched community (Activated sludge) | carbon felt | 469 | -0.9 $^{Ag/AgCl}$ | 1689 | 23 | 54 | 11.35 | (Su et al., 2013) |
| | | 1738 | -1.1 $^{Ag/AgCl}$ | 10444 | 141 | 90 | 7.60 | |
| *S. ovata* | carbon rod | 208 | -0.4 $^{SHE}$ | 1385 | 45 | 89 | 5.04 | (Nevin et al., 2010) |
| | carbon cloth | 71 | | 360 | 12 | 76 | 6.63 | (Zhang et al., 2013) |
| | chitosan [e] | 475 | | 2748 | 89 | 86 | 5.81 | |
| | Au [e] | 388 | | 2172 | 71 | 83 | 6.00 | |
| | CNT–cotton [e] | 220 | | 1224 | 40 | 83 | 6.04 | |
| *S. sphaeroides* | carbon rod | 17 | | 63 | 2 | 39 | 9.07 | (Nevin et al., 2011b) |
| *C. ljungdahlii* | | 31 | | 141 | 5 | 68 | 7.27 | |
| Reference processes | | | | | | | | |
| Mixed community [f] | - | - | - | - | 430 | n.a | - | (Nie et al., 2008) |
| *A. woodii* [g] | - | - | - | - | 7400 | n.a | - | (Demler & Weuster-Botz, 2011) |
| Cativa™ process | - | - | - | - | 67680000 [h] | n.a | - | (Sunley & Watson, 2000) |

a) Calculated assuming a non-limiting anode poised at 1V vs. SHE
b) Due to unknown surface area, current density and areal production rate could not be calculated
c) Maximum reported
d) Average reported
e) Carbon cloth is modified with these materials
f) 2 stage glucose fermentation with gas recirculation
g) Pressurized $H_2$ fermentation
h) Rate reported in lab experiments

n.a.: not available
- : not applicable

# CHAPTER 7: GENERAL DISCUSSION

# General Discussion

In this work various aspects (anode materials, anode processes and cathode design [chapter 2-4]) of the use of a bioelectrochemical system in a sediment setting have been investigated. Next to that useful applications for the generated current have been further developed (making $H_2O_2$ and reducing $CO_2$ to organics [chapter 5-6]).

These aspects can be placed in the three frameworks as described in the introduction, (i) the energy/wastewater business (chapter 2, 4 and 5), (ii) the product business (chapter 5 and 6) and (iii) niche applications (chapter 3 and 5) (Figure 1.5). However, it can be easily seen that these 'markets' are not strictly separated e.g. chapter 5 deals with enhanced treatment of wastewater and makes a product ($H_2O_2$ or by extension, clean water). As can be seen in chapter 3, a small amount of power can be generated from rice paddies while making impacting on greenhouse gas emissions. The main message of this work is thus: to make a BES a commercial technology for future generations, integrated applications should be found or niche markets need to be targeted where high value can be created to off-set high investment costs of the complex reactor systems and the relatively slow rate of biological conversion.

## 1. Perspectives for plant- and sediment-BES

### 1.1 Developments on the anode of a Plant-MFC



**Electrode configuration and current collection in sediment based systems**

The anode of a bioelectrochemical system has been widely studied and the processes governing anode current generation are reasonably well understood (Lovley, 2006; Lovley, 2008). However, the step to applying all this knowledge in practice is hampered by further development of the whole reactor system, including separator, cathode, electrochemical control, hydrodynamic patterns and integration of all this knowledge. This is especially the case for sediment based MFCs. Whereas small scale, low power applications have been developed and deployed in environments (Shantaram et al., 2005; Tender et al., 2008), the challenge remains to develop a larger scale (modular) system that can deliver more power than required for powering remote sensing equipment. With the current status of sensing and sending equipment, electronic engineering to include capacitors and voltage convertors is key to achieve workable current and power for the devices. Sensors powered by BES required occasionally 3 W for about 1s to send the recorded data and about 5-18 mW continuous to drive the recording equipment (Tender et al., 2008). As described in chapter 2 and 3, current collection in sediment anodes is of major importance to maximize the conversion of organic matter into an electrical current. For sediment based systems containing plants that are not harvested, felt, rods or other solid interconnected materials are a good option. Applications involving plants, that are harvested or where agricultural practices are applied, benefit more from loose material but in that situation current collector design is of high importance. An unexplored concept is the use of carbonized nets or carbon fibres in conjunction with the granular material to collect the current. The importance of a good current collector/electrode system, is also illustrated in chapter 4, where at high organic carbon concentrations methanogens were able to outcompete current producing microorganisms, possibly indicating that surface area of the anode material was limiting. However,

other studies have shown that even when anode compartments are operated in a packed bed configuration, methanogenic activity cannot be excluded from the anode (Freguia et al., 2008).

A further enhancement on electrode configuration and system design can be made by extending the work presented in chapter 3, where a buried cathode was explored. Upon using techniques that have been used for making gas diffusion electrodes or pressing a conductive layer on a membrane, a membrane electrode assembly can be created with minimal electrode distance and an efficient current collection system (Liu & Logan, 2004; Pant et al., 2010b). This design is aimed at maximizing power output and can be applied in wetlands, green roofs and greenhouse horticulture. A possible design configuration, combining ideas from chapter 4 and 5 can be seen in figure 7.1. Depending on the electrochemical control of the system it can be used to function as a BOD sensor or it can be used to produce power, $H_2O_2$ or remove nutrients (nitrate or sulphate) at the cathode.

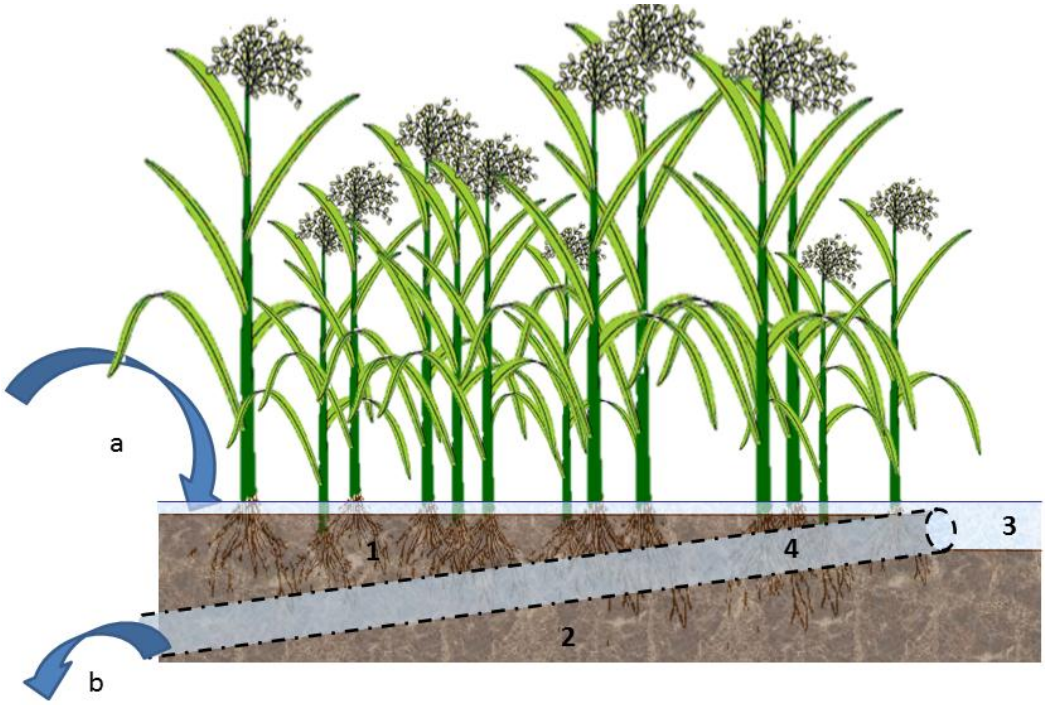



***Figure 7.1****: integrated wetland concept. a: wastewater influent b: clean water out. Processes 1: rhizodeposition and nutrient uptake. 2: anode organic carbon oxidation 3: closed box for passive re-aeration of filtered water 4: cathodic process, optionally, the cathode can be divided in sections and/or poised at a set potential to allow nitrate removal, oxygen reduction for power production or oxygen reduction for $H_2O_2$ production for disinfection purposes.*

**Anode microbial community and electron transfer mechanisms in plant-MFC anodes**

Little is known about the indigenous rhizosphere microbial community with respect to current generating capabilities. The general concept for the anode process is the fermentation of longer chain organic components into small organic components which are subsequently oxidized at the anode by dedicated current generating microorganisms (Timmers et al., 2011; Timmers et al., 2012b). This metabolic network is very similar to the one found in various anaerobic environments (Liesack et al., 2000; Angenent et al., 2004). However, plants have evolved with specific microbial

communities on their roots for a mutual benefit. This rhizosphere microbiome offers opportunity for mining of microorganisms that are able to interact with plants and stimulate organic carbon release as well as using this organic carbon directly to generate a current. Possible locations where these functions can be found are for example iron rich (salty) mangrove marshes, iron rich rice paddies and metal contaminated sites that are being remediated by means of phytoremediation. However distinction of functions per microbial partner, as described before, also holds true for these environments. Therefore genomic approaches can be taken to refrain from testing numerous species for electrode reducing activity.

Another track of mining for interesting organisms are the electron shuttle producing microorganisms of which *Pseudomonas aeruginosa* is an example (Rabaey et al., 2005b). Little is known about the influence of electron shuttles in a (plant) sediment MFC. Appling electron shuttles or microorganisms that produce these components can potentially increase the effective range of the anode electrode i.e. *Geobacter sulfurreducens* or similiar organisms will take care of direct electrode reduction with organic carbon that is available close to the anode while *P. aeruginosa* or *Shewanella oneidensis* type organism are able to oxidize organic carbon away from the anode but can still interact with it by means of the electron shuttles they produce. As these electron shuttles are non-specific they can take up electrons from other sources (e.g. microorganism) as well (Pham et al., 2008b). Drawback of the non-specificity of the electron shuttles is the fact that they can be re-oxidized by other compounds other than the anode electrode and they need to migrate to the anode to be effective in enhancing current generation. Therefore the effectiveness of this proposed “bio-augmentation” needs to be tested thoroughly. Application of electron shuttle molecules in soils can also be effective against methane production in anaerobic soils, as they are able to redirect electron flows. Besides actively produced shuttle molecules, indigenously present soil humic acids might also aid in electron shuttling in anaerobic soils (Bond & Lovley, 2002).



Gaining a better understanding of electron transfer pathways at a larger distance from the electrode is not only beneficial towards current production in sediment based BES but can also help in bioremediation challenges in contaminated anoxic soils (Aulenta et al., 2010; Lovley, 2011a).

**Organic carbon donors in anodes of Plant-MFCs**

During this work (chapter 3) and in other published works long start-up times (weeks to months) were encountered while studying anodes with plants (De Schamphelaire et al., 2008; Helder et al., 2010; Timmers et al., 2010; Helder et al., 2013b). Moreover, little to no volatile fatty acids or small organic molecule were detected (chapter 3 and 5). Therefore it seems likely that root exudation is of less importance compared to rhizodeposition processes for driving current production in a plant-MFC. These results validate the model developed by Timmers et al. (2011) who argued that maximization of root biomass would be most beneficial for long term performance of a plant-MFC. In the overall application of plant-MFCs, the long start-up times do not impact the performance that much as these devices are envisioned to be operated at timescales of years (Helder et al., 2013a). However the effect of anode processes (organic carbon and electron removal and local acidification) on exudation and rhizodeposition is still not well understood and offers good perspectives for further research.

## 1.2 Cathodes in a sediment-MFC

In this work various cathode processes and designs have been studied ($O_2$ reduction for power, chapter 4, $O_2$ reduction for disinfection, chapter 5, and $CO_2$ reduction for organics production chapter 6). Although the microbiology of oxygen reduction was not studied in detail in this work, other studies have elucidated various microbial communities of $O_2$ reducing cathodes. Interestingly not one or a few isolated species can be identified as major players (to the extent of *Geobacter* spp. in the anode process) in the oxygen reduction catalysis or as essential for start-up of oxygen reducing biocathodes. Another unanswered question is what the effect of $H_2O_2$ is on the start-up dynamics of oxygen reducing biocathodes. A putative link between $H_2O_2$ peroxide production and the start-up of an oxygen reducing biocathode has already been suggested but never been fully elucidated (Clauwaert, 2009). Understanding these dynamics can help to create a faster and more reliable start-up of oxygen reducing biocathodes. Other biocathode start-up dynamics can also benefit from this understanding as the trace concentrations of oxygen might still be present after anaerobic media preparation.

Cathodic processes in sediment based systems are difficult to control and depend for a large extent on the amount of (in)organic carbon available. In seawater settings organic carbon concentrations are low as well as carbon fixation rates. Therefore it is unlikely that cathode fouling by means of heterotropic microorganisms or algae will occur. However, in marsh, estuarine and wetland based systems, the organic carbon concentration is higher as well as $CO_2$ fixation rate by autotrophic organisms. This can lead to cathode fouling, i.e. formation of a heterotrophic biofilm or deposition of organic detritus. Growth of algae has the benefit of producing locally high oxygen concentrations that are beneficial for efficient power generation but long term algae coverage leads to dead organic matter formation with subsequent blockage of the cathode. This was readily observed in various rice-MFCs (non-published results; Figure 7.2). For marsh and wetland based MFCs peroxide production at the cathode might be a beneficial process as cathode 'fouling' will limit cathode performance on the long term. However the effectiveness of keeping a cathode free of organic deposits during long term operation by means of $H_2O_2$ needs to be verified. At this point a more reliable method would be to replace the cathode periodically with a new or regenerated active (bio) cathode.

***Figure 7.2**: Example of a cathode from a rice-MFC at the end of the growth season, showing complete coverage of the surface with algae and other biomass.*

Placement of the cathode in plant-sediment MFCs is a key consideration as it will influence internal resistance of the sediment MFC (chapter 4). Not only the internal resistance is influenced but also cathode reactions can be severely hampered by wrongly placed cathodes. When placed on the soil

level, close to the roots, roots can penetrate the cathode electrode and start depositing organic carbon (not shown). Although it has been suggested that radial oxygen loss by rice roots is able to sustain the cathodic reaction (Chen et al., 2012), it is more likely that long term performance of the cathode will be hampered by root penetration and accompanying rhizodeposition processes.

Overall, the future for (plant)-sediment systems is foreseen along two distinct paths of development. The first path is the integration of the (plant)-sediment MFC with electrical and electronics engineering disciplines so that reliable low power applications can be developed for areas where surface area is of low cost. Scalable, modular systems can be equipped with for instance a cell balance system to minimize detrimental effects of one failing module on the complete system (Andersen et al., 2013). The second path of development is creating of additional benefits next to power production. These system are meant to be applied in more densely populated areas where land is in higher demand. For example, integration of a plant-MFC in a green roof adds benefits such as isolation, water retention and environmental services (i.e. higher biodiversity) to low power production (Helder et al., 2013a). Whether these extra attributes warrant the high investment costs (in price but also in environmental footprint) of the plant-MFC needs to be thoroughly evaluated.

## 1.3 Interactions between plants and electrodes for monitoring bioproduction

The work around placement of the anode in a plant-MFC has mostly been confined to closed containers. Placing anode vertically in the soil in concentric circles resulted in an decrease in electrochemical activity with increasing distance from the plant (Chiranjeevi et al., 2012). Combining their work with an impedance measurement of the rhizosphere (Repo et al., 2005) should be able to provide non-destructive long-term follow-up of below ground root biomass of a large number of plants. This can be particularly useful when integrating carbon felt electrodes in rockwool slabs for monitoring root development in horticulture settings. Possibly even nutrient uptake or concentration of the feed can be monitored by calibrating impedance spectra with known reference situations (Dominguez-Benetton et al., 2012).

## 1.4 Interactions between plants and electrodes for enhanced bioproduction

In all the work described on plant-MFC research, no attention has been paid to the effect of an electrical field on plant productivity and health. However the earth's magnetic field induces also electric field and a gravity field (Weisenseel & Meyer, 1997). Moreover cell membranes of all living cells provide, by means of a potential gradient, a driving force for energy conservation (Matsuoka et al., 1999; Richardson, 2000). In this context, it is interesting to understand whether the application of an electric field or an electrical current in the rhizosphere of plants has on their physiology, either good or bad (Aon & Cortassa, 1989; Hamada et al., 1992; Stenz et al., 1998; Robinson & Messerli, 2003). As the applied electrochemical parameters, plant species used and results differ widely, standardized analysis should be put forward as advocated in the introduction of this work. All research considering plants as a supplier of reducing equivalents has shown that grasses were the most favourable group to use (De Schamphelaire et al., 2008; Strik et al., 2008; Helder et al., 2010; Bombelli et al., 2013). Grasses have been used to a limited extent for this type of research but seem to offer a good candidate as they are able to withstand the electrochemical conditions of an anode.

In a preliminary work, related to this thesis, it was noticed that applying an electrical field of 0.2 V $cm^{-1}$ on the rhizosphere of tomato seedlings (*Solanum lycopersicum L. cv. 'Dirk')* resulted in growth

enhancement of seedlings that received low nutrient concentrations (¼ or ½ standard solution) as compare to seedlings that only experienced the natural gravitation, magnetic and electrical fields (Olyslaegers, 2012). The effect was only noticed in the autumn/winter season and not in spring/summer. This in combination with the widely varying results in literature warrants further research. Several hypotheses can be put forward: 1) electrical fields induce enhanced migration of nutrients to provide better access to them. 2) pH gradients induce nutrient release from the soil/sediment. 3) the electrical field or current is able to modulate (expression of) nutrient uptake mechanisms. 4) the electrical field or current is able to alter the direction of root growth (Desrosiers & Bandurski, 1988), thus enhancing movement of the root to places where more nutrients are available. 5) the electrical field or current is able to stimulate the indigenous rhizosphere microbial community to modulate interaction with the plant. These are only some avenues that need to be explored to understand the relation between electromagnetic fields and biological growth patterns.

Following along the path of making use and understanding natural electrochemical gradients, the mixing of fresh and salt water holds a tremendous amount of potential energy (Cusick et al., 2012; Logan & Elimelech, 2012). The potential gradient is not only present at the water surface where rivers flow into the oceans but similarly in the subsurface where groundwater meets seawater. Placing electrodes in, for instance, polluted salt marshes offers the opportunity to produce *in situ* hydrogen and oxygen gas by means of reverse electrodialysis. Contaminant removal in the anaerobic subsurface is usually limited by electron donors and acceptors. Therefore the *in situ* produced hydrogen and oxygen can serve as electron donor and acceptor to enhance microbial catalysed contaminant removal (Guimarães et al., 2010). This proposal shows similarities to combining a permeable reactive barrier in a polluted groundwater flow to monitor heavy metal contamination and removal (Williams et al., 2010).



## 2. Perspectives for microbial electrosynthesis

### 2.1 Biological cathodes in BES for production processes

Cathodes in MFC are used for the purpose of reducing a final electron acceptor at a high potential in order to maximize energy production of the whole system. However, research interest is shifting towards using the cathode as the 'main electrode' of the BES where it is potentiostatically controlled and the anode reaction is of minor importance and is oxidation of organics in wastewater or $O_2$ evolution (Rabaey & Rozendal, 2010). Initially applications for this type of process operation were envisioned in producing gaseous fuels ($H_2$ and $CH_4$) and removing environmental contaminants such as oxidized nitrogen compounds ($NO_3^-$, $N_2O$), metal contaminants and dyes. These applications can be placed in the wastewater or niche application category. The other category, 'making a product' has received much attention lately in the domain of microbial electrosynthesis (Chapter 1 and 6). As the proof of concepts emerge, whether it is $CO_2$ to organics or conversion of substrate organics into product organics, a critical evaluation of the usefulness of using a biofilm based, electrode driven reduction is indeed the better option. As proposed in chapter 6, *in-situ* hydrogen production via water electrolysis might be the option with rates that are able to create an economically sound process.

The interaction of an electroactive microorganism with a cathode still needs to be elucidated. While hypotheses of electron transfer have been put forward (Rosenbaum et al., 2011), no solid evidence

for any one of them has been presented yet. Direct electron transfer is suggested to be the most likely process during $CO_2$ reduction to organics (Nevin et al., 2010; Nevin et al., 2011b). However no evidence based on cyclic voltammograms in combination with deletion mutant studies has been presented thus far. As suggested in chapter 6, hydrogen production at the cathode, even in small undetectable amounts is a valid alternative theory. Cyclic voltammograms of cathodes incubated with homoacetogens have shown a decrease in the energy needed for the hydrogen evolution reaction after a certain period. This was also observed in mixed culture studies, suggesting that microorganisms have the ability to change the microenvironment near the cathode and thus making $H_2$ production possible that could not be detected in abiotic controls (Villano et al., 2010; Marshall et al., 2013a; Su et al., 2013). This is an interesting route to explore as studies involving mixed culture fermentations have benefitted from cathodic current addition. For example, conversion of glycerol (a waste product from the biodiesel industry) resulted in differing microbial community profiles and product outcomes depending whether the current was turned or off. Supply of hydrogen gas did not have this effect (Dennis et al., 2013).

Interaction with the cathode electrode is also a physico-chemical interaction. This is another important aspect of microbial electrosynthesis. Microorganisms on a cathode rarely develop a dense biofilm by themselves. Strategies to create a good biofilm on cathodes include feeding substrate organics without polarizing the surface or using the electrode as an anode and subsequently switching polarity. These strategies and others, such as surface modifications need to be further explored to understand the interaction of a microorganism or mixed culture with a polarized surface.



Next to electron supply, nutrients are essential for microbial growth and metabolism. Omitting essential nutrients such as nitrogen can minimize growth. This is a valid strategy when catalysis of a certain reaction is not coupled to growth of the biocatalyst. Preferably biocatalysts are used that do not couple growth to product formation as this leads to a loss of efficiency, the need to dose essential nutrients and the formation of excess biomass.

## 2.2 Systems biology and metabolic engineering for improving microbial interaction with an electrode

Systems biology is an integrated approach to understand metabolic pathways in microorganisms based on genomic, proteomic and metabolomics information. Together with metabolic engineering these powerful tools offer the perspective of creating dedicated products from waste streams. This approach is finding its way into BES research slowly. For *Geobacter sulfurreducens*, expression profiles transcriptomics data are becoming available (Krushkal et al., 2007; Nevin et al., 2009) so that models of the physiology can be created (Mahadevan et al., 2011). The model organism *Escherichia coli* has been used for metabolic engineering studies in the anode of an MFC. *E. coli* is not a native current producer but mediated current production could be enhanced by introducing a glycerol dehydrogenase gene in the cell (Xiang et al., 2009). Next to mediated electron transfer also the direct electron transfer pathway of *Shewanella oneidensis* was successfully brought to expression in *E. coli* (Jensen et al., 2010). However further tuning of the expression pathway was necessary to optimize the incorporation of the new capabilities in the existing genetic background (Goldbeck et al., 2013). This shows the need for a comprehensive understanding of the pathways that govern energy and carbon flows in microorganisms before metabolic engineering can be applied.

A metabolic model of *E. coli* for electrosynthesis already yielded valuable results. For example, trade-offs between production and growth rate were recognized, growth rate and n-butanol production increased upon current addition while for succinate production is increased at the expense of growth rate upon current addition (Pandit & Mahadevan, 2011). The first steps to understand and optimize cathodic electron transfer are already under way as the genome of *Clostridium ljundahli* (a homoacetogen) has been elucidated and tools have been developed for genetic modification of this organism have been developed (Leang et al., 2012). Although no direct electron transfer could be established in pure cultures of *Acetobacterium woodii* (Nevin et al., 2011b), the results of mixed culture studies and of chapter 6 in this work might warrant directing efforts towards creating tools for *Acetobacterium woodii* (Marshall et al., 2013a; Su et al., 2013).

Now it is clear that several bioproduction processes can be accomplished by means of microbial electrosynthesis (glycerol to 1.3 propanediol, fumerate to succinate, $CO_2$ to acetate, formate, butyrate). Future efforts need to be channelled in a direction where demand for products is high (i.e. resulting high revenue per gram of product) and electrosynthesis can provide an extra benefit as opposed to existing best practice.

## 3. Conclusion

Several aspects of bioelectrochemical systems in a sediment setting have been studied in this work. The applicability of granular carbon as an anode material was shown (**chapter 2**), however the use of this material comes at an output loss compared to more interconnected materials (felts, plates). Therefore, application of granular material is possible but needs to be validated compared to other alternatives.

In several studies the suggestion was made that sediment-MFCs are able to minimize $CH_4$-gas release from rice paddies and other anaerobic wetlands. This hypothesis was tested in **chapter 3**. It was shown that current production preceded COD build-up in the bulk of the microcosm and preceded methane emissions. However, methane release, once started could not be stopped. Therefore, it can be stated that methane emissions cannot be mitigated by use of a sediment-MFC, possibly only postponed. Moreover, low bulk COD concentrations are a key element to successful competition.

Cathode placement is key for a low internal resistance of a sediment-MFC. In **chapter 4** it was shown that burying a membrane tube as a cathode in the sediment gave better outputs compared to using a drain tube. Buried cathodes gave better results compared to top floating cathodes.

The combination of wetland wastewater treatment with $H_2O_2$ production in the cathode was useful for disinfection of wetland effluent (**chapter 5**). This work showed the proof of concept but needs to be further developed and fine-tuned in terms of flow rates and nutrient removal before it can be applied at a larger scale.

Microbial electrosynthesis of acetate from $CO_2$ and electrical current by *Acetobacterium woodii* was successful both on stainless steel and carbon cathodes. Applying a fixed current density showed higher production rates compared to a fixed potential at a similar energy input per gram of product (**chapter 6**).

# ABSTRACT

The plant-microbial fuel cell (PMFC) is one of the many possible embodiments of a bioelectrochemical system (BES). A BES is an electrochemical reactor in which whole microorganisms serve as catalysts for the anode, cathode reaction or both. At the anode, oxidation processes take place liberating electrons. These electrons are transported through an electrical circuit towards the cathode where they are used for a reductive reaction. In a fuel cell configuration, the system is able to produce electrical power from the biological conversion of organic matter from e.g. wastewater (domestic or industrial), soils and sediments at the anode. In the cathode usually (a)biotic oxygen reduction takes place. In a different approach, some electrical power can be invested in the BES to drive reactions that are under normal conditions thermodynamically unfavorable, coupled to wastewater oxidation at the anode. By doing so, various products can be produced at the cathode such as inorganics (NaOH, $H_2O_2$), gaseous fuels ($H_2$, $CH_4$) and organic molecules (e.g. acetate, 1,3 propanediol). The biological production of organic molecules from $CO_2$ or substrate molecules with electrons as a substrate is termed microbial electrosynthesis (MES).

BES are the central technology platform of this work. Therefore a literature review and guidelines for benchmarking are presented in **chapter 1**. The anode of a BES can be placed in anaerobic soils and sediment with subsequent oxidation of organic matter. The cathode will be placed in the above water layer to drive oxygen reduction to water. In this way an electrical current can be generated at remote locations by means of a sediment-MFC. This system can be enhanced by placing plants in the anode, which provide a continuous flow of organic matter to the anode by means of rhizodeposition. Up to 40% of the photosynthetic production can be excreted into the rhizosphere.

As anode materials, only interconnected materials have been considered for use in a sediment-MFC. In **chapter 2** granular carbon materials were investigated for their suitability as anode material in sediment microbial fuel cells. Eight different electrode materials (granules, felts and cloths) were examined. Felt material had an overall superior performance in terms of current density per amount of applied electrode material i.e. felt and granular anode obtained similar current densities (approx. 50–60 mA $m^{-2}$) but felt materials required 29% less material to be applied. Small granules (0.25–0.5 mm) gave the highest current density per land area compared to larger granules (1-5 mm) of the same material (55.2 ± 11.7 mA $m^{-2}$ vs. 25.2 ± 7.74 mA $m^{-2}$). Granules with a rough surface had a better performance compared to smooth granules of the same size (55.2 ± 11.7 mA $m^{-2}$ vs. 5.33 ± 7.9 mA $m^{-2}$). The different granular materials lead to a selection of distinct microbial communities for each material. Granular carbon was shown to be suitable as anode material for sediment microbial fuel cells.

**Chapter 3** explores the perspectives for application of a Sediment-MFC in cultivated areas. An example of these cultivated areas are rice paddies. These fields contain an anaerobic soil with suitable conditions for a plant-MFC. Moreover, methane ($CH_4$) release from these paddies is an important source of greenhouse gas emissions (25 times as potent as $CO_2$). Greenhouse gas production is heavily dependent on rhizosphere biogeochemical conditions (i.e. substrate availability and redox potential). It is hypothesized that by introducing a biocatalyzed anode electrode in the rhizosphere of wetland plants, a competition for carbon and electrons can be invoked between electrical current generating bacteria and methanogenic archaea. Therefore, microcosms with rice plants (*Oryza sativa*) and a sediment-MFC were studied for their impact on methane emissions.

Microbial current generation was initially able to outcompete methanogenic processes when the bulk matrix contained low concentrations of organic carbon. When interrupting the electrical circuit or supplying an excess of organic carbon, methanogenic metabolism was able to outcompete current generating metabolism. The qPCR results showed hydrogenotrophic methanogens were the most abundant methanogens present, while mixotrophic or acetoclastic methanogens were hardly detected in the bulk rhizosphere or on the electrodes. Competition for electron donor and acceptor, as opposed to acidification of the anode, was likely the main driver to lower methane emissions. Overall, electrical current generation with a BES is an interesting option to control $CH_4$ emissions from wetlands but needs to be applied in combination with other mitigation strategies to be successful and feasible in practice.

Cathode placement is one of the few aspects that can be engineered in a sediment-MFC. Ideally the cathode is positioned as close as possible to the anode to obtain a low internal resistance. In **chapter 4**, a tubular cathode was placed in the sediment and its performance compared to a cathode in the above water layer. A membrane tube showed the highest and most stable current (87 ± 2.5 mA $m^{-2}$) and power density (38 ± 2.1 mW $m^{-2}$). A drain tube cathode wrapped with geo-textile generated less current (47 ± 21.5 mA $m^{-2}$) and power (14 ± 8.8 mW $m^{-2}$). However, the total system had a lower overall internal resistance (1.1 Ω $m^2$ for the drain tube vs. 2.6 Ω $m^2$ for the membrane tube). Cathodes placed in the overlying water layers showed in general a poor performance in comparison to the bottom cathodes. An elongated tubular cathode can be constructed as a membrane electrode assembly and deployed by means of horizontal drilling over larger surface areas, without extensive digging.

As indicated in the literature review, several higher value applications compared to power production are studied for BES. To apply this concept in combination with a wetland, **chapter 5** is dedicated to the study of a highly-loaded constructed wetland for wastewater treatment (up to 44 ± 21 g COD $m^{-2}$ $d^{-1}$) linked to a BES. The anode was able to produce a current from the wetland effluent up to 3.5 A $m^{-2}$ (maximum 62% anodic efficiency) but was limited in the supply of oxidizable organic carbon. Hydrogen peroxide production at the cathode was tested at various current densities. A maximum production rate of 2.7 g $m^{-2}_{electrode}$ $h^{-1}$ in wetland effluent was obtained (4 hours at 10 A $m^{-2}$, 41% cathodic efficiency). The hydrogen peroxide (0.1%) was used to disinfect wetland effluent (1 h contact time resulted in plate counts of <75 CFU $ml^{-1}$). The flow rates can be tuned to produce various water qualities (e.g. for irrigation or use as potable water). Thus by linking a BES to a constructed wetland, water treatment with controllable disinfection can be assured.

Another possible application for the production of higher value outcomes from a BES is by means of microbial electrosynthesis. The key example is the production of acetate from $CO_2$ in the cathode. Stainless steel and carbon cathodes were compared in **chapter 6** at a fixed current density using *Acetobacterium woodii* as a biocatalyst. Acetate production rates of 536 ± 226 mg $m^{-2}$ $h^{-1}$ for a steel cathode and 340 ± 131 mg $m^{-2}$ $h^{-1}$ for a carbon cathode were achieved. These rates can be estimated to amount to 200 g $m^3$ $d^{-1}$ (assuming 20 $m^2$ $m^{-3}$). Operation at a fixed current density increased production rates per unit projected surface at similar energy investment per gram of product (4.8-7.5 kWh $g_{acetate}^{-1}$) as compared to production at set potentials in other studies. Whereas further improvements are needed to make this process competitive with existing approaches, these results show that *in situ* hydrogen production with steel has the potential to rapidly transfer electrons to planktonic cells.

Based on the results presented in the research chapters of this work, several proposals for future work are discussed in **chapter 7**. These include an integrated study on the impact of combining the findings of chapter 2,4 and 5 to create a power producing wetland with an optimized design. This can be expanded to study methane emissions from wetlands in combination with the drain tube cathode which can provide additional $O_2$ in the rhizosphere. Disinfection or (bio)electrochemical enhanced nutrient extraction of spent water can be an interesting avenue to explore within greenhouse horticulture or aquaculture. However, a suitable niche should be found for the application of a plant MFC to maximize its benefits. This includes maximizing the output of the cathode as illustrated in chapter 6. However, the combination of a plant-MFC with MES seems unlikely at the moment but plenty of opportunities exist for further exploration of these emerging technology concepts in the framework of the biobased economy.

# SAMENVATTING

De plant microbiële brandstof cel (PMBC) is een van de vele mogelijke vormen van een bioelektrochemisch systeem (BES). Een BES is een elektrochemische reactor waarin hele micro-organismen dienst doen al katalysator voor een reactie aan de anode, de kathode of beide. Aan de anode vind een oxidatie plaats waarbij elektronen worden vrijgesteld. Deze elektronen worden door een elektrisch circuit naar de kathode geleid waar ze gebruikt worden voor een reductie reactie. In een brandstof cel opstelling is het systeem in staat om elektrisch vermogen te produceren van de biologische omzetting van organisch materiaal van b.v. afvalwater (huishoudelijk of industrieel), bodems en sedimenten aan de anode. In de kathode vind meestal (a) biotische zuurstof reductie plaats. In een alternatieve aanpak kan een beetje energie aan het systeem worden toegevoegd om reacties door te laten gaan die onder normale omstandigheden thermodynamisch niet mogelijk zouden, gekoppeld aan afvalwater oxidatie aan de anode. Door dit te doen kunnen allerlei verschillende producten aan de kathode geproduceerd worden zoals anorganische stoffen (NaOH, $H_2O_2$), brandstoffen ($H_2$, $CH_4$) en organische moleculen (b.v. azijnzuur of 1,3 propaandiol). De biologische productie van organische moleculen van $CO_2$ of van organische substraten met elektronen als een substraat wordt microbiële elektrosynthese (MES) genoemd.

Bioelektrochemische systemen zijn het centraal platform van dit werk. Daarom wordt een literatuur onderzoek en enkele handreikingen voor een referentiekader gepresenteerd in **hoofdstuk 1**. De anode van een BES kan geplaatst worden in anaerobe bodems en sedimenten met vervolgens oxidatie van het aanwezige organisch materiaal. De kathode wordt in de bovenstaande waterlaag geplaatst waar zuurstof reductie naar water plaatsvindt. Op deze manier kan een elektrische stroom geproduceerd worden op afgelegen locaties door middel van een sediment-MBC. Dit systeem kan versterkt worden door een plant in de anode te plaatsten. Deze zorgt voor een constante aanvoer van organisch materiaal door middel van rhizodepositie. Tot 40% van de fotosynthetische productie kan uitgescheiden worden in de rhizosfeer.



Als anode materiaal zijn enkel goed verbonden materialen overwogen voor gebruik in een sediment-MBC. In **hoofdstuk 2** werd granulair koolstof materiaal onderzocht omtrent de geschiktheid als anode materiaal in sediment microbiële brandstof cellen. Acht verschillende electrode materialen (korrels, vilt en stof) werden bestudeerd. Vilt gaf in het algemeen de beste prestaties per hoeveelheid aangebracht elektrode materiaal. Vilt en korrel materiaal gaven uiteindelijk dezelfde stroomdichtheid (ongeveer 50–60 mA $m^{-2}$) maar voor het vilt was 29% minder materiaal nodig. Kleine korrels (0.25–0.5 mm) zorgden voor de grootste stroomsterkte per land oppervlak in vergelijking met grotere korrels (1-5 mm) van hetzelfde materiaal (55.2 ± 11.7 mA $m^{-2}$ vs. 25.2 ± 7.74 mA $m^{-2}$). Korrels met een ruw oppervlak hadden een betere prestatie in vergelijking met gladde korrels van dezelfde grootte( 55.2 ± 11.7 mA $m^{-2}$ vs. 5.33 ± 7.9 mA $m^{-2}$). De verschillende korrel materialen zorgden voor een selectie van onderscheidende microbiële gemeenschappen voor elk materiaal. Korrelig koolstof bleek geschikt als anode materiaal voor sediment microbiële brandstof cellen.

**Hoofdstuk 3** verkent de perspectieven voor toepassing van een sediment-MBC in gecultiveerde gebieden. Een voorbeeld van zulke gecultiveerde gebieden zijn rijstvelden. Deze velden bestaan uit anaerobe bodems met geschikte omstandigheden voor een plant-MBC. Methaan emissies van deze velden is een belangrijke bron van broeikas gas emissies (25 keer zo sterk als $CO_2$). Broeikas gas

productie is sterk afhankelijk van rhizosfeer biochemische omstandigheden (d.w.z. substraat beschikbaarheid en redox potentiaal). Het wordt verondersteld dat door het introduceren van een biogekatalyseerde anode in de rhizosfeer van moeras planten, een competitie voor koolstof en elektronen op gang gebracht kan worden tussen stroom producerende bacteriën en methanogene archaea. Daarom werden microcosmossen met rijstplanten (*Oryza sativa*) en een sediment-MBC bestudeerd omtrent de impact op methaan uitstoot. Microbiële stroom productie was in het begin in staat om de competitie te winnen van methanogene processen wanneer de bulk rhizosfeer lage concentraties van organische koolstof bevat. Als de stroomkring werd onderbroken of als er veel organische koolstof aanwezig was, was het mogelijk voor het methanogene metabolisme om de competitie te winnen van stroom producerende bacteriën. Resultaten van qPCR analyse lieten zien dat waterstof consumerende methanogenen het meest aanwezig waren terwijl azijnzuur consumerende methanogenen en mixotrofe methanogenen nauwelijks gedetecteerd werden in de bulk en op de elektroden. Competitie voor elektron donor en acceptor, in tegenstelling tot verzuring van de anode, was waarschijnlijk de belangrijkste oorzaak van de verlaagde methaan uitstoot. Concluderend, stroom productie met een BES is een interessant optie om CH4 uitstoot van moerassen te beperken maar moet worden toegepaste met andere strategieën om succesvol en mogelijk te zijn in de praktijk.

Plaatsing van de kathode is één van de weinige aspecten dat geregeld kan worden in een sediment-MBC. Idealiter wordt de kathode zo dicht mogelijk tegen de anode geplaatst zodat lage interne weerstanden ontstaan. In **hoofdstuk 4** werd een buisvormige kathode in het sediment geplaatst en zijn prestatie werd vergeleken met kathodes in the bovenliggende waterlaag. Een buis gemaakt van membraan gaf de hoogste en meest stabiele stroomdichtheid (87 ± 2.5 mA $m^{-2}$) en vermogensdichtheid (38 ± 2.1 mW $m^{-2}$). Een drainagebuis kathode gewikkeld in geo-textiel produceerde minder stroom (47 ± 21.5 mA $m^{-2}$) en vermogen(14 ± 8.8 mW $m^{-2}$). Echter het totale systeem had een lagere interne weerstand (1.1 Ω $m^2$ voor de drainage buis vs. 2.6 Ω $m^2$ voor het membraan). Kathodes die in de bovenliggende waterlaag werden geplaatst lieten over het algemeen een slechte activiteit zijn in vergelijking met de kathodes in de bodem. Een lange buis kan uitgevoerd worden als een membraan/elektrode paar en aangebracht worden door middel van horizontaal boren, met weinig graafwerkzaamheden.

Zoals aangegeven in het literatuur onderzoek, worden alreeds verschillende toepassingen van BES met meer toegevoegde waarde in vergelijking met stroom onderzocht. In **hoofdstuk 5** werd dit concept in combinatie met een wetland toegepast om het wetland hoger te belasten voor afvalwater behandeling (tot 44 ± 21 g CZV $m^{-2}$ $d^{-1}$)met koppeling aan een BES. De anode van de BES was in staat een elektrische stroom van het water uit het wetland te produceren, tot 3.5 A $m^{-2}$ (maximum 62% anodische efficiëntie) maar werd gelimiteerd in de toevoer van oxideerbaar organische koolstof. Waterstof peroxide productie aan de kathode werd getest met verschillende stroomsterktes. Een maximale productie snelheid van 2.7 g $m^{-2}_{electrode}$ $h^{-1}$ in wetland gefilterd afvalwater werd behaald (4 uur op 10 A $m^{-2}$, 41% kathodische efficiëntie). Waterstof peroxide (0.1%) werd vervolgens getest voor disinfectie van hetzelfde water (1 u contact tijd resulteerde in plaat tellingen van <75 KVE $ml^{-1}$). De debieten kunnen worden geoptimaliseerd om verschillende waterkwaliteiten te produceren (b.v. voor irrigatie of drinkwater). Door het koppelen van een BES aan een wetland is het mogelijk om afvalwater te behandelen met controleerbare disinfectie.

Een andere mogelijkheid voor de productie van meer toegevoegde waarde door een BES is door middel van microbiele elektrosynthese. Het bekendste voorbeeld is de productie van azijn van $CO_2$ in de kathode. Roestvast staal en koolstof kathodes werden vergeleken in **hoofdstuk 6** bij een vastgelegde stroomdichtheid en met *Acetobacterium woodii* als de biokatalysator. Azijn productiesnelheden van 536 ± 226 mg $m^{-2}$ $u^{-1}$ voor een roestvast staal kathode en 340 ± 131 mg $m^{-2}$ $u^{-1}$ voor een koolstof kathode werden bereikt. Deze snelheden komen neer op 200 g $m^3$ $d^{-1}$ (20 $m^2$ $m^{-3}$ aangenomen). Vastleggen van de stroomsterkte verhoogde productiesnelheden per geprojecteerde oppervlak maar liet de energie nodig voor de productie van een gram product (4.8- 7.5 kWh $g_{acetate}^{-1}$) gelijk aan de waarden behaald tijdens studies met vastgelegde potentiaal. Ondanks dat verder ontwikkeling nodig is om dit precies competitief te maken met bestaande processen, deze resultaten tonen aan dat *in situ* waterstof productie met staal de mogelijkheid biedt om efficiënt elektronen over te dragen op micro-organismen in oplossing.

Gebaseerd op de resultaten van de onderzoekshoofdstukken in dit werk worden verschillende voorstellen voor verder onderzoek besproken in **hoofdstuk 7**. Onder andere een geïntegreerd onderzoek naar het effect van de kombinatie van de resultaten van hoofdstuk 2, 4 en 5 om een vermogen producerend wetland te creëren met een geoptimaliseerd ontwerp. Dit kan verder worden uitgebreid naar verdere studie van methaan uitstoot van wetlands in combinatie met een drainage buis kathode welke extra $O_2$ kan inbrengen in de rhizosfeer. Disinfectie of (bio)elektrochemische nutriënt extractie van gebruikt water kan een interessant pad zijn voor onderzoek in het kader van teelt in kassen of in de aquacultuur. Echter, een geschikte niche moet gevonden worden voor een plant-MBC om zijn voordelen maximaal te benutten. Dit houdt in dat de productie aan de kathode gemaximaliseerd moet worden, zoals geïllustreerd in hoofdstuk 6. Echter de combinatie van een plant-MBC met MES lijkt op het moment nog onwaarschijnlijk maar er bestaan genoeg mogelijkheden voor verder onderzoek van deze opkomende technologische concepten in het kader van de bio-economie.

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# CURRICULUM VITAE

## Personal Information

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Born: June 16th 1982, Emmen, The Netherlands
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## Academic background

05/2013- **Researcher ERC project "Electrotalk"**
Promoter: Prof. Korneel Rabaey
Ghent University, Belgium

05/2009-12/2013 **PhD Candidate in Applied Biological Sciences**
Ghent University, Belgium
Funding: EU-FP7 "PlantPower"
Promoters: Prof. Nico Boon, Prof. Korneel Rabaey, Prof. Willy Verstraete
Thesis: *Optimizing the plant microbial fuel cell:diversifying applications and product outputs.*

09/2001-01/2009 **MSc./BSc. Biotechnology. Specialisation: Cellular/ molecular**
Wageningen University, Wageningen, the Netherlands
MSc. Thesis Microbiology: *Propionate oxidation in a MFC: current production and community analysis*
Laboratory of Microbiology, Wageningen University in Wageningen
Promoter: Prof. Fons Stams, Dr. Jeanine Geelhoed

Internship Microbiology: *Pseudomonas aeruginosa in a Microbial Fuel Cell: A characterization of mutants.*
The Angenent Lab, Department of Energy, Environmental & Chemical Engineering, Washington University in St. Louis, USA.
Promoter: Prof. Lars Angenent

MSc. Thesis Virology: *Translation initiation of Tomato Spotted Wilt Virus.*
Laboratory of Virology, Wageningen University in Wageningen.
Promoter: Dr. Richard Kormelink, Christina Geerts-Dimitreadou

BSc-Minor in Strategic Management

09/2000-05/2001 **High school**, East High School in Waterloo, IA, USA.

09/1994-05/2000 **High school**, Carmel College (former KDC) in Emmen.

**Other relevant experience**

Organizing committee's

09/2012 1st European meeting of the international society for microbial electrochemical technologies (EU-ISMET). Gent, Belgium. Aimed at stimulating interaction with the general bioelectrochemistry community, ± 200 attendants.

09/2012 1 day electrochemistry workshop linked to the EU-ISMET conference, ± 65 attendants.

02/2010 1st international PlantPower Symposium. Gent, Belgium. Aimed at dissemination of results from the EU-FP7 PlantPower project and stimulating interaction with the general (bio)electrochemistry community, ± 60 attendants.

05/2005 May 5th Committee. Wageningen, The Netherlands. Organizing festivities for one out of 14 music stages to celebrate Dutch liberation day, ± 5000 attendants.

Tutoring experience

10 Master students (1 yr. thesis; Bio-Engineering and Environmental Sanitation, Ghent University, Gent)

1 Bachelor Student (6 month thesis; Biomedical Laboratory Technology, KaHo St. Lieven, Gent)

Supervisor for 3 incoming visitors (> 3 month visit)

Teaching experience

12/2012
12/2011
12/2010 Computer exercises: biofilm modelling & wastewater treatment plant modelling in: "Biotechnologial processes". Ghent University, Gent, Belgium

10/2008 Teaching part of a labclass: "Global Biogeochemical Cycles" at the Wageningen University in Wageningen, the Netherlands

12/2007
05/2008 Teaching part of a practical course to college freshmen and high school seniors about the basics of a Microbial Fuel Cell at Washington University in St. Louis, USA

03/2007 Supervision of student workgroups for the course 'Cell biology and Health' at the Wageningen University in Wageningen, the Netherlands

## Scientific contributions

### A1 publications:

**Arends, J.B.A.**, Boon, N., Rabaey, K. 2013. Microbial electrosynthesis of acetate on carbon or steel cathodes: Is there a difference? *Submitted*

**Arends, J.B.A.,** Vandenhouwe, S., Verstraete, W., Boon N., Rabaey, K. 2013. Enhanced disinfection of wastewater by combining wetland treatment with bioelectrochemical $H_2O_2$ production. *Submitted*

De Vrieze, J.*, Gildemyn, S*., **Arends, J.B.A***., Vanwonterghem, I., Verbeken, K., Boon, N., Verstraete, W., Tyson, G.W., Hennebel, T., Rabaey, K. Biomass retention by means of a bioelectrochemical system enhances stability in anaerobic digestion. *Submitted*
*these authors contributed equally.

**Arends, J.B.A**., Speeckaert, J., Blondeel, E., De Vrieze, J., Boeckx, P., Verstraete, W., Rabaey, K., Boon, N. Greenhouse gas emissions from rice microcosms amended with a plant microbial fuel cell. *In Press: Applied Microbiology & Biotechnology*. DOI: 10.1007/s00253-013-5328-5

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Desloover, J., **Arends, J.B.A**., Hennebel, T., Rabaey, K. 2012. Operational and technical considerations for microbial electrosynthesis. *Biochemical Society Transactions*, **40**(6).

Zhang, Y., **Arends, J.B.A**., Van de Wiele, T., Boon, N. 2011. Bioreactor technology in marine microbiology: From design to future application. *Biotechnology Advances*, **29**(3), 312-321.

Guimarães, B.C.M., **Arends, J.B.A**., Van der Ha, D., Van de Wiele, T., Boon, N., Verstraete, W. 2010. Microbial services and their management: recent progresses in soil bioremediation technology. *Applied Soil Ecology*, **46**(2), 157-167.

Venkataraman, A., Rosenbaum, M., **Arends, J.B.A.,** Halitschke, R., Angenent, L.T. 2010. Quorum sensing regulates electric current generation of Pseudomonas aeruginosa PA14 in bioelectrochemical systems. *Electrochemistry Communications*, **12**(3), 459-462.

### Other publications:

**Arends, J.B.A.**, Van Denhouwe, S., De Vrieze, J., Boon, N., Verstraete, W., Rabaey, K. 2013. Integration of wetland wastewater treatment with disinfection via bioelectrochemical H2O2 production. *Communications in Agricultural and Applied Biological Sciences*, **78**(1), 173-7.

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# DANKWOORD

Tot slot nog een woordje van dank aan allen die mij op enigerlei wijze hebben gesteund, tijdens mijn onderzoeksavontuur van de afgelopen jaren. Finally a well meant thank you for everybody who has supported me during my research adventures the past year.

I would like to start with thanking all EU-citizens for paying their taxes so that the financial support of this work and the EU-FP7 framework project 'PlantPower' was warranted. For more information on how your money was spent: www.plantpower.eu.

Dit werk kon niet worden voltooid zonder effectieve begeleiding. Ik had het geluk drie fantastisch verschillende promotoren tot mijn beschikking te hebben: Willy, bedankt voor al je energie, je wilde, grootse ideeën en je gedrevenheid mensen verder te laten kijken dan hun neus lang is. Korneel, je kwam later bij het project en bracht goede ideeën, gedegen vakkennis en veel nieuwe inspiratie mee hartelijk dank hiervoor. Ik waardeer het zeer dat ik nog wat langer mag blijven. Nico, jij hebt het project alle vier de jaren van dichtbij meegemaakt en was de stabiele factor in de begeleiding waar ik altijd terecht kon. Daarnaast was je een zeer waardevol adres voor de uitdagingen omtrent de moleculair biologische vraagstukken. Hartelijk dank voor alle ondersteuning.

Naast de Proffen van LabMET ben ik ook mijn dank verschuldigd aan Prof. Boeckx, Prof. Steppe en Prof. Geelen. Deze experts hebben mij op verschillende wijzen verder geholpen met mijn onderzoek, hartelijk dank hiervoor.

To the jury members, I really enjoyed the discussions with you on this specific work and on more general matters over the past years.

Ontwikkelen van ideeën en scherpen van je gedachten kan je alleen doen maar is veel leuker en geeft meer resultaat als je het samen doet. Gelukkig kent LabMET vele medewerkers die hieraan mee willen en kunnen werken, ieder mijn zijn eigen kennis en expertise. Allen, hartelijk dank hiervoor! Joachim, een speciale vermelding voor u aangezien we lange tijd met z'n tweeën het fameuze BES-cluster gevormd hebben. Ondanks dat ik soms moeite had u te verstaan/begrijpen, heb ik afgelopen 4 jaar uw aanwezigheid zeer gewaardeerd. I would also like to thank Jo and Carlos for helping me discover the intricate world of anaerobic digestion.

Tijdens mijn onderzoek heb ik veel studenten en bezoekers mogen begeleiden met hun onderzoek. Dit zorgde iedere keer weer voor verrassingen en interessante vragen en discussies. Sommige projecten hebben mij direct verder geholpen met mijn onderzoek, andere waren zeer uitdagend en preliminair. Bedankt voor deze leuke tijden Jeroen, Jarne, Sanne, Stijn, Sylvia, Sara, Arne, Frederiek-Maarten, Evelyne, Jonas, Tom. Gracias, tak, Sebastia, Nuria, Jorge, Anders, Mick for your interesting visits to LabMET, I wish you all the best with your further work.

Contacts outside the lab are of great importance to change one's perspective. I would like to thank all the PlantPower project partners for their contribution to the great project meetings at various locations throughout Europe. Also other great scientists that I have met during various conferences made sure of very motivating and interesting encounters, Prof. Rosenbaum, Prof. Angenent, and many more of the MFC/BES community, thanks.

Onderzoek vindt vaak vooruitgang door de stille werkers op de achtergrond. Daarom mijn zeer hartelijke dank voor de nodige ondersteuning op het gebied van ICT, microbieel, moleculair en analytisch werk en reactor bouw aan al het technisch personeel van LabMET; Tim, Jana, Greet, Siska, Mike, Robin, Rita. Naast praktisch labowerk is de administratie van het geheel niet te onderschatten. Christine en Regine, hartelijk dank mij terzijde te staan met raad en daad, vooral tijdens het voldoen aan de vele verzoeken van de verschillende administratieve instanties en het afronden van (tussentijdse) verslagen

Naast het vele werk in het labo spendeert een doctoraal onderzoeker tegenwoordig veel tijd aan zijn bureau. Dan is het wel zo prettig dat de mensen om je heen voor een goede (werk)sfeer zorgen. Bedankt voor de gezellige en serieuze momenten en activiteiten Charlotte, Echo, Rosemarie, Jo, Arnout, Annelies, Marlies, Linde, Eline, Eva en de vele andere bezoekers. Ontspanning tijdens de lunch is ook van levensbelang, Karen en vele anderen bedankt voor alle gezellige lunches, soms serieus, maar vaak een klein uurtje vol goede/slechte grappen waarna we weer met goede moed aan de slag konden gaan.

Af en toe ontspannen en weer met de voeten op de grond staan is van groot belang. Gelukkig kwamen er met enige regelmaat familie en vrienden uit het 'hoge noorden' langs zodat ik mijn gedachten wel moest verzetten. Bedankt voor deze leuke weekenden en onverwachtse bezoekjes tijdens dienstreizen (Thierry, Annebeth, Floris, Ed). Anderzijds stonden de bezoekjes aan 'de Wageningse vrienden', ondanks de soms lange reistijden, altijd garant voor de nodige onbezorgde uren.

Zonder familie sta je nergens, daarom een groot woord van dank voor mijn ouders, Jan en Josetta, voor de vrijheid en ondersteuning die jullie mij altijd hebben gegeven om mijn eigen weg te kunnen zoeken.

De familie Toetenel en Van de Sande mogen ook niet onvermeld blijven. Ongeveer 10 jaar kom ik al bij jullie op bezoek en word ik altijd hartelijk ontvangen met kritische vragen. Bedankt voor alle gezelligheid en leuke gesprekken. Viveka, bedankt voor de vele probeersels die je hebt gemaakt om dit werk te voorzien van een mooie omslag.

Milou, je wil er niks van weten maar toch sta je hier als slot vermeld in dit werk. Ik schrijf dit terwijl jij ligt te slapen (zoals zo vaak de laatste tijd). Ik ben heel blij dat je samen met mij aan het avontuur in België bent begonnen en hier ook je eigen draai hebt kunnen vinden. Ik ben trots op jou. Veel heb ik aan je te danken; ontspanning, reality checks, kritische vragen, het vinden van Benoit en Louise ….. en nog veel meer. Ik ga graag met jou nog vele avonturen tegemoet!